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Conservation and Ecology of the Yellow-Blotched Sawback (*Graptemys flavmaculata*)

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CONSERVATION AND ECOLOGY OF THE YELLOW-BLOTCHED SAWBACK

(GRAPTEMYS FLAVIMACULATA)

by

William Winsyl Selman II

Abstract of a Dissertation
Submitted to the Graduate School
of The University of Southern Mississippi
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy

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ABSTRACT

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The role of species conservation is becoming increasingly important due to the mounting pressures from humans on habitats and populations of organisms. This is particularly evident in riverine ecosystems throughout the world where the human demand for freshwater resources is increasing, and consequently, the number of imperiled aquatic organisms is also growing. The seven chapters of this dissertation primarily focus on the Yellow-blotched Sawback, *Graptemys flavimaculata*, an endangered riverine turtle that is endemic to the Pascagoula River system of southeast Mississippi, USA. At the population level, we aimed to study the impacts of Hurricane Katrina on two study populations within the Pascagoula River system, while also determining likely causes of population decline related to the storm. On a larger scale, we sought to determine the current distribution and abundance of *G. flavimaculata*, as well as another similar species (Pascagoula Map turtle, *Graptemys gibbonsi*), throughout the Pascagoula River system. At the individual/population level, we wanted to better understand the basking ecology of the species by analyzing basking behavior throughout the active season. In addition, the role of human recreation on the species was studied by measuring behavioral (basking) and physiological (stress hormone levels and shell

condition) responses of turtles to human activities. Lastly, we aimed to determine the level of connectivity of *G. flavimaculata* throughout the Pascagoula River system by measuring levels of genetic differentiation among sampled populations.

Our results indicated that Hurricane Katrina had a significant negative impact on coastal populations, whereas an upstream population was not similarly impacted. We suspect that following the hurricane, water quality was likely compromised, leading to an impact on prey items and subsequent impacts on turtle populations. Our distribution/abundance surveys found both *Graptemys* species in new localities, in smaller drainages than earlier studies, and generally in higher population densities than previously recorded; our coastal population, however, did not rebound in the three years following Hurricane Katrina. Our basking ecology study found that turtle basking behavior was dependent on the season, time of day, and the sex of the individual. Also, we found differences among the sexes in basking structures used, but little correlation was found with population-level basking and measured environmental temperatures. Our recreational impacts study found significant impacts of boating activities on our coastal population relative to our inland population. The former had significantly shorter individual basking durations and significantly lower population-level basking percentages, while also having poorer physiological condition as measured by shell condition. However, we found no correlation between recreational activities and baseline/corticosterone stress response measures. Further, larger/slower boats disturbed significantly higher numbers of turtles relative to smaller/faster boats. This is significant as registered boats in Jackson County, MS (county of our disturbed site) have increased 300% over the last 22 years. Results of our genetic analysis indicated that genetic differentiation was significant among almost all sampled populations with isolation by distance likely being the driving mechanism; the Escatawpa population was found to be the most genetically distinct population, with differentiation likely derived from a historical isolation from the main river populations. We hope that this research, conducted on multiple scales, will provide quality information for conservation planners/managers to preserve viable turtle populations and intact riverine habitat throughout the Pascagoula River system into the future.

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CHAPTER I

GENERAL INTRODUCTION

A great deal of public and conservation attention has been given to the global decline of species worldwide, particularly vertebrate animals such as birds and mammals. The conservation of reptile species has garnered relatively little attention even though there are documented declines, or even extinctions, of many terrestrial and aquatic reptilian species worldwide. The primary factors contributing to these declines include the loss of habitat, negative impacts from invasive species, environmental contaminants, diseases, collection for food/pet trade, and potentially, climate change (Gibbons et. al 2000). Within the class Reptilia, 28 percent of species assessed by the by the World Conservation Union are currently considered critically endangered, endangered, or threatened (IUCN 2008). However, within the Reptilia, turtles (order Testudines) are considered one of the most endangered vertebrate taxonomic groups with 131 of the 212 species (62%) assessed considered critically endangered, endangered, or threatened, with an additional eight species considered extinct or extinct in the wild. In comparison, 12 percent of birds (highest endangered Order: 61 percent- Sphenisciformes [penguins]) and 21 percent of mammals (highest endangered Order: 48 percent- Primates) are considered critically endangered, endangered, or threatened (IUCN 2008). Therefore, even though reptiles, including turtles, have had little conservation attention, as well as fewer funds for conservation work relative to birds or mammals, these data indicate that they are in no less trouble than other fauna that receive more attention.

The Southeastern United States is considered to be one of the most diverse turtle fauna regions worldwide, harboring over 10% of the world's turtle species (Folkerts

1997). This region, however, is not immune to species endangerment, and over 60 percent of the turtle species in the southeastern U.S. are considered imperiled and at risk for declines, for reasons similar to other reptilian species (collection for harvest/pet trade, habitat destruction/degradation/fragmentation; Buhlmann and Gibbons 1997). Several turtle species of the southeastern U.S. have been directly targeted by humans over the last century for food (Alligator Snapping Turtle [*Macrochelys temmenckii*] and Gopher Tortoises [*Gopherus polyphemus*]) or for the pet trade (Box Turtle species [*Terrapene* sp.], Bog Turtle [*Glyptemys muhlenburgii*], and Map Turtles [*Graptemys* sp.]; Thorbjarnarson et al. 2000). In many of these cases, the harvest/collection pressure on turtle populations was unsustainable due to the unique life history of turtles. Turtles have a unique life history because they are long-lived organisms, they delay reproductive maturity, have slow growth, and there is a high rate of juvenile and hatchling mortality (Iverson 1991; Congdon et al. 1993, 1994); therefore, adult turtles in the population are the most important life history stage. The most important factor that is intrinsically linked to the decline of many turtle species in the southeastern U.S., as well as worldwide, is the loss, degradation, or fragmentation of habitat (Mitchell and Klemens 2000). In the Southeastern U.S., this is evident with river turtle fauna, particularly Map Turtles (Genus *Graptemys*), whose habitats are threatened by human modification/alteration including channelization, impoundments, de-snagging (removal of trees and deadwood from the river channel), pollution, or excess sedimentation (Moll and Moll 2004).

Map turtles (*Graptemys*) are highly aquatic turtles that primarily inhabit riverine systems. They are also one of the most diverse groups of turtles, encompassing almost

25 percent of the North American turtle fauna (12 of 50 total species; Ernst and Lovich 2009). Furthermore, 8 of the 12 species are endemic to single river systems that drain into the Gulf of Mexico, from the Guadalupe River in Texas to the Apalachicola River in Florida (Moll and Moll 2004); it is hypothesized that the most likely factor accounting for speciation in this group is a series of sea level fluctuations that extended from the Pliocene to late Pleistocene era that served as an isolating mechanism for river turtle 'populations' (1.8 million years ago - 10,000 years ago; Lamb et al. 1994). Many *Graptemys* are also considered imperiled, threatened, or endangered due to the combination of river drainage endemism, their specialization to riverine ecosystems, and the degradation of river systems (via channelization, sedimentation, and impoundments; Buhlmann and Gibbons 1997, Ernst and Lovich 2009).

Within this group, the yellow-blotched sawback (*Graptemys flavimaculata*) is considered to be one of the most imperiled *Graptemys* species (http://www.iucn-tftsg.org/trouble/; accessed 3 December 2009). It is imperiled due to its endemicity to the Pascagoula River system, small maximum area occupied within the river system (56.7 km^2), a large number of potential threats, and observed population declines (Selman and Jones, *in press*). Following population declines in the 1980's, this led the U.S. Fish and Wildlife Service to list the species as federally threatened in 1991 (U.S. Fish and Wildlife Service 1991). Further, relatively little was known about the life history and ecology of *Graptemys flavimculata* before it was listed, which thereafter prompted several studies to investigate home range/seasonal habitat use (Jones 1994, 1996), reproduction/nesting ecology (Horne et al. 2003), basking/nesting behavior with emphasis on human disturbance (Moore 2003, Moore and Seigel 2006), diet (Seigel and Brauman 1994), habitat associations (Lindeman 1998, 1999), and seasonal steroid hormone cycles (Shelby et al. 2000, Shelby and Mendonça 2001).

The objective of this dissertation was to use a multifaceted approach to determine the ecology and current conservation status of *Graptemys flavimaculata*. 1.) To understand population level dynamics in response to Hurricane Katrina, we monitored populations following the storm to determine if there were any impacts to turtle populations. 2.) On the species level, we sought to determine the current distribution and abundance of the species, as well as a similar *Graptemys* species (*Graptemys gibbonsi*, Pascagoula Map Turtle), throughout the Pascagoula River system by surveying turtles at road-stream crossings, surveying selected portions of the river system by canoe, and at several sites using mark-resight methods to estimate population sizes. 3.) We also observed individual and population level basking behavior to determine the affects of sex, season, time of day, basking structure type, and environmental temperatures on individual basking behavior/population basking frequency. 4.) To determine the impact of recreational boating in the lower Pascagoula River on turtles, we monitored basking behavior at a disturbed and undisturbed site, while also monitoring physiological responses, shell condition and corticosterone (stress hormone) levels, in response to these disturbances. 5.) Lastly, we used population genetic analyses (via microsatellite marker data) to determine the connectivity of *G. flavimaculata* populations throughout the Pascagoula River system, as well as to determine significant management units if appropriate. By using multiple approaches and methods, we hope that our results will inform wildlife managers to make better decisions with regards to the conservation of this species and its sole habitat, the Pascagoula River system.

CHAPTER II

THE IMPACTS OF HURRICANE KATRINA ON A POPULATION OF YELLOW-BLOTCHED SAWBACKS (*GRAPTEMYS FLAVIMACULATA*) IN THE LOWER PASCAGOULA RIVER

Abstract.—The Yellow-blotched Sawback (*Graptemys flavimaculata*) is a riverine turtle that is endemic to the Pascagoula River system of southern Mississippi, USA. Population declines led to Federal listing as a threatened species in 1991, with the most robust population inhabiting the Lower Pascagoula River near Vancleave, MS (approx. 24 river km from the Pascagoula River mouth). We conducted a mark-recapture study of this population during the spring and summer of 2005-2006. On 29 August 2005, Hurricane Katrina hit the Mississippi Gulf Coast, the location of our study site. On 13 October 2005, we conducted a one-hour preliminary visual survey by boat through the study area and we identified 8 individuals that we paint-marked prior to Katrina's landfall. This demonstrated that at least some of the 49 previously-marked individuals remained in the study area. Later, we conducted more extensive mark-resight surveys within the same 2.0 km section of river in October of 2005-2006. The population estimate for 2006 was significantly lower than the 2005 population estimate for the same stretch of river, suggesting that numbers substantially decreased during the year following the hurricane. Of the plausible explanations for this pattern, the available evidence most strongly supports a real decline in population, presumably due to the long-term impact of Hurricane Katrina. Possible reasons for such a long term effect include hurricane induced 1) saltwater intrusion and 2) low levels of dissolved oxygen with direct impacts

on individuals or indirect impacts on the prey populations (e.g., gastropods and other aquatic macroinvertebrates). Follow-up surveys are planned to further investigate these influences on the long-term population trends of *G. flavimaculata*.

INTRODUCTION

The Yellow-blotched sawback (*Graptemys flavimaculata*) is a freshwater aquatic turtle that is endemic to the Pascagoula River system of southern Mississippi, USA (Figure 2.1). It occurs only in the Pascagoula River and its tributaries, including the Leaf, Chickasawhay, and Escatawpa Rivers (Cliburn 1971; U.S. Fish and Wildlife Service 1993; Selman and Qualls 2006; Ernst and Lovich 2009). *Graptemys flavimaculata* also inhabit portions of the Bouie River, Okatoma Creek, Bluff Creek, Tallahala Creek, and Bogue Homa Creek (Selman and Qualls 2006). Population declines led to its Federal listing as a threatened species in 1991 (U.S. Fish and Wildlife Service 1991), but it had been listed as an endangered species by the State of Mississippi since the 1970's (Mississippi Natural Heritage Program, 2003). *Graptemys flavimaculata* are obligate freshwater turtles that predominantly occur in main river channels and larger tributaries. However, Jones (1996) noted that some female *G. flavimaculata* moved overland to nearby cypress ponds, while males occurred in nearby oxbow lakes and flooded timber following overbank flooding. Except for such anecdotal observations, we know very little about the behavioral response of these and other riverine turtles to natural flooding events, especially the massive flooding associated with storm surge and heavy rains from a major hurricane.

Figure 2.1. Male Yellow-blotched sawback (*Graptemys flavimaculata*) from the Lower Pascagoula River. The species is characterized as having a narrow head with yellow stripes, a yellow blotch on each pleural scute, and a pronounced vertebral keel in males and a less pronounced ridge in females (Photograph by Mary Perez).

The most robust population of *G. flavimaculata* exists in the lower Pascagoula River (Figure 2.2) in the vicinity of Vancleave, MS, about 24 river km from the Pascagoula River mouth (McCoy and Vogt 1980, Stewart 1989). During the spring and summer of 2005 and 2006, we focused a trapping and mark-resight study on this population using visual surveys during October to estimate population size in the study area. On 29 August 2005, Hurricane Katrina made land-fall on the Mississippi Gulf Coast, including our study site. The study area experienced high winds of > 190 km/hr, and a 3.7 to at least 4.6 m storm surge. In addition to the surge, the river reached 5.0

Figure 2.2. One female (bottom) and three male Yellow-blotched sawbacks (*Graptemys flavimaculata*) basking on a snag in the Lower Pascagoula River. This is a common sight in the Lower Pascagoula River where densities are the highest within the range of *G. flavimaculata*. The habitat in this section of the Pascagoula River is characterized as a large, moderate to high flow river with ample sunlight and many snags available for basking (Photograph by Mary Perez).

gauge m and remained at > 3.0 m for 13 days (Figure 2.3), with normal river levels between 0.9 to 1.2 m and flood stage occurring at 4.6 gauge m (Michael Runner, USGS; pers. comm.). The only available river stage data is from Graham's Ferry gauging station, which is 26 river km upstream from our study site. This was the only station online throughout Hurricane Katrina. Because of the northern location of this station, these numbers are likely underestimates for flooding at our study site. Thus, Hurricane Katrina offered the opportunity to study the response of *G. flavimaculata* to one of the most severe natural disasters occurring in this drainage during recorded history.

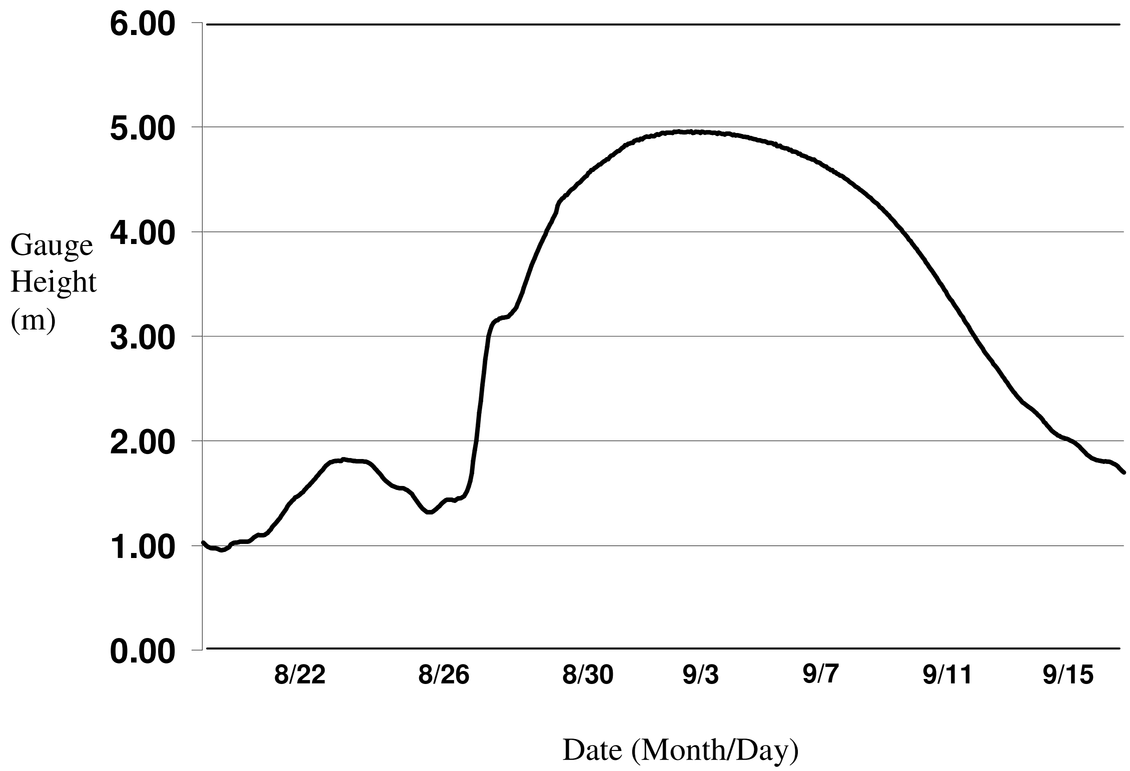


Figure 2.3. Hurricane Katrina stage data from the Graham's Ferry hydrologic station. The location of the gauging station is approximately 26 river kilometers north of the lower Pascagoula River sampling site (data provided by Michael Runner, USGS) and it was the only gauging station to stay online throughout Hurricane Katrina.

MATERIALS AND METHODS

Study Site.—We conducted our study on the Pascagoula River, Jackson County, Mississippi. We used mark-resight surveys on 2.0 km of the west channel of the Pascagoula River between Poticaw Bayou (31° 30.948 / 088° 37.002), north to the end of the mainstem of the Pascagoula River (30° 30.948 / 088° 36.184). This portion of the Pascagoula has moderate to high flow rates and abundant submerged and emerged deadwood snags. Many bayous, oxbow lakes, and side channels occur within this stretch of river floodplain.

Sampling Technique.—During April through October of 2005 and 2006, turtles were trapped by slightly submerging open topped basking traps (made of 3/4" PVC coated crawfish wire; The Fish Net Company, Jonesville, LA) to turtle basking structures. Traps varied in size from 56 x 46 x 31 cm to 122 x 61x 25 cm and we used nails and cotton twine to fasten traps to logs or branches known to be *G. flavimaculata* basking sites. We used a maximum of 15 traps during a trap-day and checked each trap every hour. We occasionally moved traps if turtles became trap wary. We also captured turtles opportunistically by hand and with a dip net.

Marking and measurements of captured turtles.—We marked captured individuals on the carapace (second and third vertebral scutes) with a nontoxic, nonpermanent, waterproof, tree marking spray paint (Aervoe® Lead-Free Fluorescent Glo Spray Paint) for subsequent mark-resight surveys. These marks allowed subsequent visual identification of sighted turtles, but did not allow visual determination of individual identity. Additionally, we obtained straightline carapace and plastron lengths (nearest mm) and body mass (nearest 5 g) for all captured turtles. We released all turtles at their point of capture after the paint mark on their shell had dried.

Population estimates.—We conducted a one-hour preliminary visual survey by boat on 13 October 2005, following Hurricane Katrina. We did not include this data in our analysis. Thereafter, we made three mark-resight surveys during October and early November 2005 and 2006, within two weeks of the initial capture and first paint-marked turtle in the population (to assure paint marks were not lost). We paint-marked at least 10 turtles prior to implementing visual count surveys to ensure a sufficient number of marked turtles available in the population. We conducted mark-resight surveys during optimal basking times (1000-1500 hr) under mostly sunny to sunny conditions. We performed all surveys by walking along the banks or sandbars and located, identified, and counted marked and unmarked turtles using a 60mm, 10-45x spotting scope with tripod (R.L. Jones, pers. comm., Shively 1999). Each survey of the 2.0 km stretch took approximately 3 hours to complete. We used the program NOREMARK (White 1996) to estimate population sizes. We used this program because we did not uniquely mark turtles and it also accounted for additional marked individuals being added to the population between survey intervals.

Body Condition Analyses.—We used morphometric data to assess body condition (mass relative to length) before and after Hurricane Katrina. To examine whether or not body condition (mass relative to length) of *G. flavimaculata* was lower following Hurricane Katrina, we used one-factor ANCOVAs, with before versus after the hurricane as a factor, plastron length the covariate, and body mass as the dependent variable (using a Log Mass transformation for females to provide a better linear fit). Plastron, rather than carapace length tends to be a more accurate measure of overall body length for these turtles, because injury/wear to the marginal scutes introduces error variation to carapace length measurements (W. Selman, unpubl. data). This analysis was done separately for male and female turtles, spanning three monthly trapping samples before (June, July, August 2005) and three following the hurricane (October 2005, April and May 2006).

Additionally, given the relatively small size of each monthly sample, lack of samples over the winter months following the hurricane (November through March), and likely normal variation in body condition over seasons (all of which would limit our statistical power), we also compared body condition of males and females for October

2005 and 2006, immediately after Hurricane Katrina and one year thereafter. A one factor ANCOVA, similar to the previous analysis, was used except year (October 2005 or October 2006) was considered the factor.

RESULTS

Population estimate results.—During our initial boat survey, we identified 8 of 49 turtles (16%) that were paint-marked prior to Hurricane Katrina at the locations where we originally marked them. Thereafter, we conducted three mark-resight surveys on 16, 18, 20 October 2005 using newly marked individuals with a different paint-marking scheme than before the hurricane. These surveys provided a population estimate of 1204 (786-2077, 95% CI) turtles in the 2.0 km stretch of river, or 602 turtles/km (Table 2.1).

We implemented three more mark-resight surveys on this stretch of river during fall 2006 (30 October; 8, 11 November). Our 2006 population estimate was 641 (481-923, 95% CI) turtles or 320.5 turtles/km (Table 2.1). The 2006 estimate was significantly lower than the 2005 population estimate (Z = 3.489, $P = 0.0003$; Seber 1982). The *G. flavimaculata* population decreased by 47% (281.5 turtles/km) between October 2005 and 2006.

Body condition analysis.—These analyses revealed no significant differences in body condition between the three monthly samples before versus after the hurricane for females ($F_{1,33} = 3.52$, $P = 0.07$) or males ($F_{1,54} = 1.87$, $P = 0.18$) and no significant factor by covariate interaction terms for females ($F_{1,38} = 3.89$, $P = 0.06$) or males ($F_{1,54} = 0.29$, $P = 0.59$). The covariate was significant in both analyses ($P < 0.001$), with body mass increasing with plastron length.

Table 2.1. NOREMARK population estimates for 2005 and 2006 for *G. flavimaculata* at the lower Pascagoula study site. The number 'marked in population' reflects the number of turtles marked within the study site and the 'marked seen' refers to the number of marked individuals spotted during the corresponding visual survey.

Site	**Survey Date**	**Marked in Population**	**Marked Seen**	**Total Seen**	**Pop. Estimate (w/ 95 % CI)**
Pascagoula	**2005**				
River	16 Oct	17	3	231	
	18 Oct	24	1	244	1204
	20 Oct	24	9	244	(786-2077)
	2006				
	30 Oct	20	6	236	
	8 Nov	24	10	233	641
	11 Nov	24	7	185	(481-923)

The other one-factor ANCOVAs also revealed no significant difference in body condition between October samples in each year for females ($F_{1,11} = 2.04$, $P = 0.18$) or males ($F_{1,38} = 0.25$, $P = 0.62$), and no significant factor by covariate interaction terms for females ($F_{1,11} = 0.09$, $P = 0.76$) or males ($F_{1,38} = 1.56$, $P = 0.12$). The covariate was also significant in both of these analyses ($P < 0.001$), with body mass increasing with plastron length.

DISCUSSION

Our observations suggest that this population survived the storm without major losses due to direct mortality. Several reports of massive fish kills following the hurricane noted no evidence of turtle mortality, thus supporting our observations (Buck 2005; Lynn McCoy, pers. comm.). Further, our initial boat survey found previously marked individuals that remained within the same stretch of river we originally marked them, indicating that some individuals maintained or restored their prior home ranges. However, we conducted our preliminary boat survey 45 days after Hurricane Katrina so we could not discern how these turtles remained in the same areas after the hurricane or returned. One possible explanation of this apparent home range fidelity could be that during periods of flooding, the turtles took advantage of the calmer environment that is available in flooded bottomland forest or side channels, in contrast to the main river channel, similar to what has been observed during non-hurricane flooding events (Jones 1996, W. Selman, per obs.). Once river levels began to subside, turtles could then have moved laterally back into the main channel of the river in the same area they had been prior to the high-water event. Alternatively, if the hurricane displaced individuals downstream or upstream, long-range linear movements to return to their home ranges may explain our results. Jones (1996) noted some long-range movements in which individuals covered long distances (>5 km) in less than one week.

The 2006 estimate suggests a substantial decrease (47%) in the number of *G. flavimaculata* in the year following Hurricane Katrina. We have no comparable baseline data (using similar mark-resight methods) prior to Hurricane Katrina to compare population trends before and after the storm, but our surveys clearly indicate a significant population decline over the subsequent year.

Our estimate one month post-Katrina (602/km) was one of the highest recorded population estimates of *G. flavimaculata* per river kilometer (41.4/km - Stewart 1989;

370/km, 269/km, 72.8/km, 58/km using multiple methods - Jones 1994). This suggests that our population estimate of 2005 may be artificially high due to congregation of turtles from upstream and downstream localities following Hurricane Katrina. The storm surge may have forced turtles northward to our study site and/or the later flooding may have washed upstream turtles downstream to our site. However, this scenario is unlikely for several reasons. First, our 2005 mark-resight survey was 48 days after Hurricane Katrina and presumably, this was enough time for displaced turtles from upstream or downstream localities to return to their respective origins. More importantly, methodologically identical population surveys of two other hurricane affected inland *Graptemys* populations (*G. flavimaculata* in the Leaf River and *Graptemys oculifera* in the Pearl River) showed no evidence of any such displacement of turtles due to flooding and prolonged high river flows during and after Hurricane Katrina (Selman and Qualls 2005, 2006). Lastly, similar to the observations by Jones (1996) during smaller floods, Sanderson (1974) observed that Hurricane Agnes had little effect on *Graptemys barbouri* movements and that individuals moved into the flooded margins of the Chipola River to avoid the fast currents of the main river channel. These observations support our contention that the high population estimate from the Lower Pascagoula River site in 2005 was not due to displacement of turtles into our study area.

Drought impacts on this population through October 2006 could also explain the population decline we observed. Most river levels during 2006 were considerably lower than in 2005 and approached 60-year lows (USGS Real-TimeWater Data, http://waterdata.usgs.gov/ms/nwis/rt). However, our comparison estimates for our two upstream sites do not support the drought-effects hypothesis. While both sites suffered from the same drought and low-river levels, we did not observe evidence of population declines during that study (Selman and Qualls 2005, 2006). Occurring in smaller, lower order streams, these northern populations were presumably equally or more subject to drought conditions and associated low river levels than the lower Pascagoula River (higher order stream) population, yet showed no negative effects. Altogether, these observations suggest that the drought had little direct effect on turtle numbers in these *Graptemys* populations.

Thus, neither short-term displacement nor drought effects adequately explain the population decline in the lower Pascagoula River. Rather, it appears that other hurricane impacts on this population of *G. flavimaculata* may explain this decline due to the coastal nature of this site. Mallin et al. (1999) noted that Hurricane Fran adversely affected benthos within the lower Cape Fear River system of North Carolina. They implicated a variety of water-quality issues (e.g., increased sedimentation, presence of contaminants, changes in dissolved oxygen levels, salinity, and an altered flow regime of the river) as possible causes. Hurricane Katrina probably caused similar changes in riverine habitats of the lower Pascagoula River. Such low levels of dissolved oxygen were documented within the Leaf River, a major tributary of the Pascagoula River (Howell 2006). Additionally, several reported fish kills in the lower Pascagoula River indicated severe water quality issues in the system (Buck 2005; MS Department of Wildlife, Fisheries, and Parks 2005; Lynn McCoy, pers. comm.). Schaefer et al. (2006) also documented fish community shifts following Hurricane Katrina within the lower Pascagoula River and lower Black Creek. Therefore, changed water quality due to Hurricane Katrina may be implicated in the *G. flavimaculata* population declines observed in the lower Pascagoula

River either by direct mortality (which was not observed) and/or by indirect means, including impacts on prey base (Seigel and Brauman 1994).

Our analyses of body condition revealed no significant differences before and after Hurricane Katrina for male or female *G. flavimaculata*. Limited sample sizes and a lack of samples in critical post-hurricane months limited our statistical power and our ability to detect any body condition shifts. Our monthly sample sizes before and after the hurricane never exceeded seven females or 21 males per month. Also, due to the severe problems with municipal/state infrastructure following Hurricane Katrina, we collected no data immediately after the storm during September 2005. We also have no data for the winter months (November 2005 thru March 2006) when turtle trapping is difficult due to the lower basking frequency during cold weather (Moore and Seigel 2006, W. Selman, pers. obs). Further, there is no published work correlating body condition to any environmental and/or seasonal changes in other highly aquatic, freshwater *Emydid* turtles (i.e. *Graptemys* or *Pseudemys*). Therefore, we have no baseline for comparison to confidently evaluate any environmental or seasonal changes in body condition.

The most convincing factor to account for the population decline at the lower Pascagoula River site was Hurricane Katrina and its potential water quality impacts on the turtles and/or their aquatic macroinvertebrate prey. Because this study of this hurricane's impacts was done opportunistically, we were unable to definitively identify the factors that caused this declines. We will continue monitoring the lower Pascagoula River population of *G. flavimaculata* through 2008 to evaluate the severity and duration of this decline and the population's potential recovery. Similar survey work is needed on other imperiled *Graptemys* species in Gulf Coast river drainages to determine baseline population estimates in preparation for future opportunities to study these phenomena and for understanding the long-term population status of these turtles (Fitch 2006; Bury 2006).

CHAPTER III

DISTRIBUTION AND ABUNDANCE OF TWO IMPERILED *GRAPTEMYS* SPECIES OF THE PASCAGOULA RIVER SYSTEM

Abstract.—Species distribution and abundance is often difficult to delineate due to species factors (i.e., crypsis, low abundance) and/or to researcher sampling techniques. Species of the genus *Graptemys* are primarily riverine turtles and have historically been subject to declines because of anthropogenic changes to river systems. Therefore, to better inform conservation efforts, we thoroughly studied the distribution and abundance of two imperiled *Graptemys* species within the Pascagoula River System, Mississippi, USA: the Yellow-blotched Sawback (*Graptemys flavimaculata*) and the Pascagoula Map Turtle (*Graptemys gibbonsi*). Turtle populations were studied in 17 counties in southeast Mississippi using four methods: mark-resight population surveys (three populations), bridge surveys (161 bridge crossings), basking density surveys without marked individuals (23 localities), and trapping (three populations). *Graptemys flavimaculata* was found to be present throughout its historical range, as well as new drainage localities; abundance in historically surveyed areas was generally higher than previous surveys reported. *Graptemys gibbonsi* was also found in many new localities and occurred in most of the drainages of the Pascagoula River system. However, *G. gibbonsi* abundance was much lower than *G. flavimaculata* throughout the Pascagoula River system and individuals were not found in several historical localities, suggesting localized extirpations. We recommend that *G. gibbonsi* should be listed as state Endangered in Mississippi and Louisiana, federally listed as Threatened, and IUCN status upgraded to Vulnerable (VU). Future conservation measures should extend to protect additional

riparian habitat throughout the Pascagoula River system and future surveys of other *Graptemys* species are warranted due to the imperiled status of this genus.

INTRODUCTION

The ability to determine the distribution and abundance of imperiled herpetofaunal species is often complicated by many factors including cryptic behaviors, low population densities, challenging habitats, and difficulty in capturing individuals for potential mark-recapture studies. Therefore, distribution records for these species are often 'patchy' and incomplete, which often poses difficulties for making conservation decisions. Accurate distributional information is increasingly important since many management decisions are now being made at a landscape level. Of particular concern are decisions that involve watersheds that are impacted due to many anthropogenic modifications (i.e., impoundments, channel alteration, poor riparian zone management) and the potential negative effects they may have on aquatic communities.

The Pascagoula River Basin is the least impacted major river system in the lower 48 United States (Dynesius and Nilsson 1994). It is also home to several endangered and imperiled aquatic species including several fish (Gulf Sturgeon [*Acipenser oxyrinchus desotoi*], Pearl Darter [*Percina aurora*], Freckled Darter [*Percina lenticula*], Crystal Darter [*Crystallaria asprella*], Alabama Shad [*Alosa alabamae*]; Ross 2001) and two imperiled *Graptemys* species, the Yellow-blotched Sawback (*Graptemys flavimaculata*) and the Pascagoula Map Turtle (*Graptemys gibbonsi*). Members of the genus *Graptemys* are highly aquatic turtles that often inhabit rivers with moderate flow rates, high numbers of deadwood snags (Lindeman 1998, 1999), and are wide enough to provide an open canopy which allows ample opportunities for aerial basking. Many of the twelve species in the genus are endemic to single or adjacent southeastern river drainages (Ernst and Lovich 2009) and therefore, many are also considered threatened or endangered due to river alterations which include impoundments, dredging, channelization, and de-snagging (manual removal of deadwood snags from a river; Buhlmann and Gibbons 1997).

Graptemys flavimaculata is a federally Threatened species that is endemic to the Pascagoula River system (U.S. Fish and Wildlife Service 1991), whereas there is currently no legal protection for *G. gibbonsi,* which occurs within the Pearl and Pascagoula River systems. Additionally, both species are considered among the top five endangered turtles in North America (IUCN/SSC Tortoise and Freshwater Turtle Specialist Group. 2007. Turtles in Trouble: North America's Most Endangered Tortoises and Freshwater Turtles. Available from http://www.iucn-tftsg.org/trouble [accessed 7 November 2008]). The last surveys for these two turtle species were conducted in the late 1970's and mid-1990's (McCoy and Vogt 1980; Lindeman 1998, 1999); the results of both of these studies suggested that populations of both species were declining.

Since the 1970's, there has been a movement to preserve the Pascagoula River system, primarily through riparian land acquisition (e.g., The Nature Conservancy) and focus on research/conservation of protected aquatic species. However, recent events seem to be antagonistic towards these efforts, including a Federal Strategic Petroleum Reserve project, refineries requesting freshwater withdrawal, proposed impoundments for recreation, and desnagging projects, all of which have the potential to alter the natural flow regime of this unique system and its aquatic communities. Because of the imperiled nature of these two *Graptemys* species, and the looming threats to the Pascagoula River system, we determined that it was critical to obtain current detailed information on the distribution and abundance of these species to document long-term changes in population distribution and/or population densities.

MATERIALS AND METHODS

Population Distribution and Relative Abundance.— We used two methods to determine the population distribution of *Graptemys flavimaculata* and *Graptemys gibbonsi*: surveys of rivers and creeks crossed by roads/highways (hereafter referred to as bridge surveys) and basking density surveys by boat/canoe. We conducted bridge surveys from August to October, 2006 and 2008, at 161 bridge crossings in 17 counties throughout the Pascagoula River basin (Figure 3.1; for exact localities see Appendix A). At each bridge survey locality, we located and identified turtles with a 60 mm, 15-45x spotting scope. From these observations, we determined presence/absence of these two basking species along with other basking turtles (*Apalone* spp.[Softshell Turtles], *Pseudemys concinna* [River Cooter], *Sternotherus carinatus* [Razorback Musk Turtle], and *Trachemys scripta* [Red-eared Slider]). We also recorded the sex of basking *Graptemys* at each bridge crossing when possible, as well as the number of each species basking to determine a relative density at each bridge survey locality. We conducted basking density surveys by boat/canoe on selected river/creek stretches within the basin to more accurately determine species presence/absence and relative abundance (Figure 3.1). During these surveys, we located basking turtles by binoculars from a canoe. When present, we walked sandbars from the upstream end to the downstream end using a spotting scope with tripod to locate basking turtles. Turtles were located more effectively by this method in comparison to locating turtles while floating downstream (Shively 1999) due to the wariness of basking turtles, particularly in creeks and rivers. We determined the presence or absence of turtles on these river stretches by these methods because of the high frequency of basking exhibited by turtles of the genus *Graptemys* (Boyer 1965, Ernst and Lovich 2009). Later, we calculated relative abundances of species as the number of basking turtles observed per river kilometer. Additionally, we recorded notable locations of turtles during boat/canoe surveys (i.e., new drainage localities or new upstream distributions) using a hand-held GPS (Garmin GPS 72).

Population estimates and trapping ratios.— During 2005-2008, we trapped *Graptemys* at three sites within the Pascagoula River system (Figure 3.1): the lower Pascagoula River (site number 1), Leaf River (site number 5), and Chickasawhay River (site number 8; trapped only in 2005 & 2006). We trapped turtles by attaching open topped basking traps (made of 3/4" PVC coated crawfish wire; The Fish Net Company, Jonesville, LA) to turtle basking structures and left them slightly submerged; traps varied in size from 56 x 46 x 31 cm to 122 x 61x 25 cm. We used nails and cotton twine to fasten traps to logs or branches known to be *G. flavimaculata*/*G. gibbonsi* basking sites. We used a maximum of 17 traps during a trap-day and checked each trap approximately every hour. We occasionally moved traps if turtles were noted to avoid the trap log. We also captured turtles opportunistically by hand and with a dip net.

Following capture, we marked individuals on the carapace (second and third vertebral scutes) with a waterproof, tree marking spray paint (Aervoe® Lead-Free Fluorescent Glo Spray Paint) for subsequent mark-resight surveys. These marks allowed visual

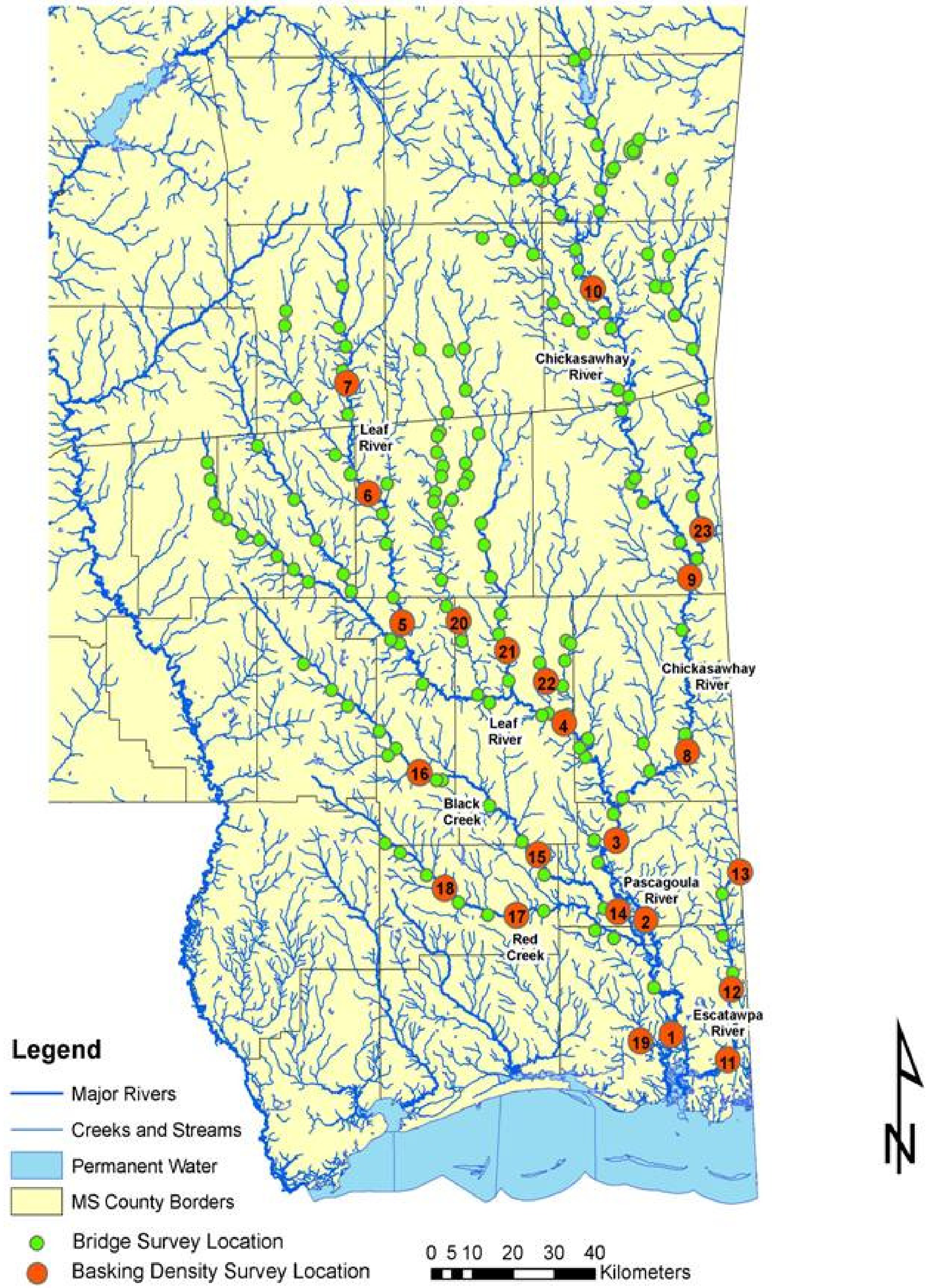


Figure 3.1. Locations of bridge and basking density surveys conducted within the Pascagoula River system, Mississippi, USA. Numbers on basking density localities correspond with site numbers in Table 3.2.

identification of marked turtles while basking, but did not allow determination of individual identity. We also determined and recorded the species and sex on all captured individuals. We released all paint-marked turtles at their point of capture after the paint mark on their shell had dried.

We made three mark-resight surveys during selected months from 2005-2008 at three locations within the Pascagoula River system (Leaf River, Chickasawhay River, lower Pascagoula River). Prior to initiating visual count surveys, we paint-marked at least 10 turtles to ensure that a sufficient number of marked turtles were available in the population. We conducted mark-resight surveys during optimal basking times (1000-1500 hr) under mostly sunny to sunny conditions during the months of April, October, or early November and each survey was completed within 3-4 hours. We performed all surveys by walking along the banks or sandbars and located, identified, and counted marked and unmarked turtles using a 60mm, 10-45x spotting scope (with tripod). Survey lengths were 2.4 river kilometers (rkm) for the Chickasawhay River site, 3.2 and 3.75 rkm for the Leaf River site, and 2.0 rkm for the lower Pascagoula River site. Also, we made surveys within two weeks of the initial capture to assure that no paint marks were lost. We used the program NOREMARK (White 1996) to estimate population sizes and this program was used because we did not uniquely mark turtles and it also accounted for additional marked individuals being added to the population between survey intervals.

We also analyzed trapping data from 2005-2008 to determine species ratios at the three trapping localities. Presumably, trapping for both *Graptemys* species would be unbiased due to similar use of the basking snags by the different species. However, juveniles were likely underestimated since they were observed during the study to bask on smaller objects closer to the bank and in shallower water, in contrast to adults that usually bask on snags away from the bank and in deeper water (Jones 1996).

RESULTS

Graptemys flavimaculata *Distribution and Relative Abundance.*—We found *Graptemys flavimaculata* to be present at all historical drainage localities to the distributional limits originally described by previous surveys (Cliburn 1971, McCoy and Vogt 1980, U.S. Fish and Wildlife Service 1993, Lindeman 1998), including within the Pascagoula River, Leaf River, Chickasawhay River, Bouie River, Escatawpa River, lower Black Creek, and lower Red Creek. New drainage localities were documented by bridge survey including Bogue Homa (Perry Co.), Bouie (Covington and Jefferson Davis Co.'s), Gaine's (Perry Co.), and Okatoma creeks (Forrest Co; Figure 3.2). Additionally, we documented new drainage localities by boat/canoe survey including within Bluff (Jackson Co.), Bucatunna (Wayne Co), and Thompson's creeks (Perry Co.). The populations within Bluff Creek and the Escatawpa River appear to be geographically disjunct from the primary population within the lower Pascagoula River due to brackish marsh between these freshwater habitats. The upstream limits for *G. flavimaculata* in the Chickasawhay River were confirmed in the vicinity of Stonewall (Clarke County, MS) by boat and bridge survey, as previously described by Cliburn (1971). However, we found by canoe survey that *G. flavimaculata* extend farther north in the Leaf River, to north of Taylorsville (Smith County, MS), approximately 25.65 rkm north of the previously known locality for *G. flavimaculata* near U.S. Hwy 84 at Hot Coffee, MS (Covington Co.; Cliburn 1971). We also documented range expansions within Tallahala Creek

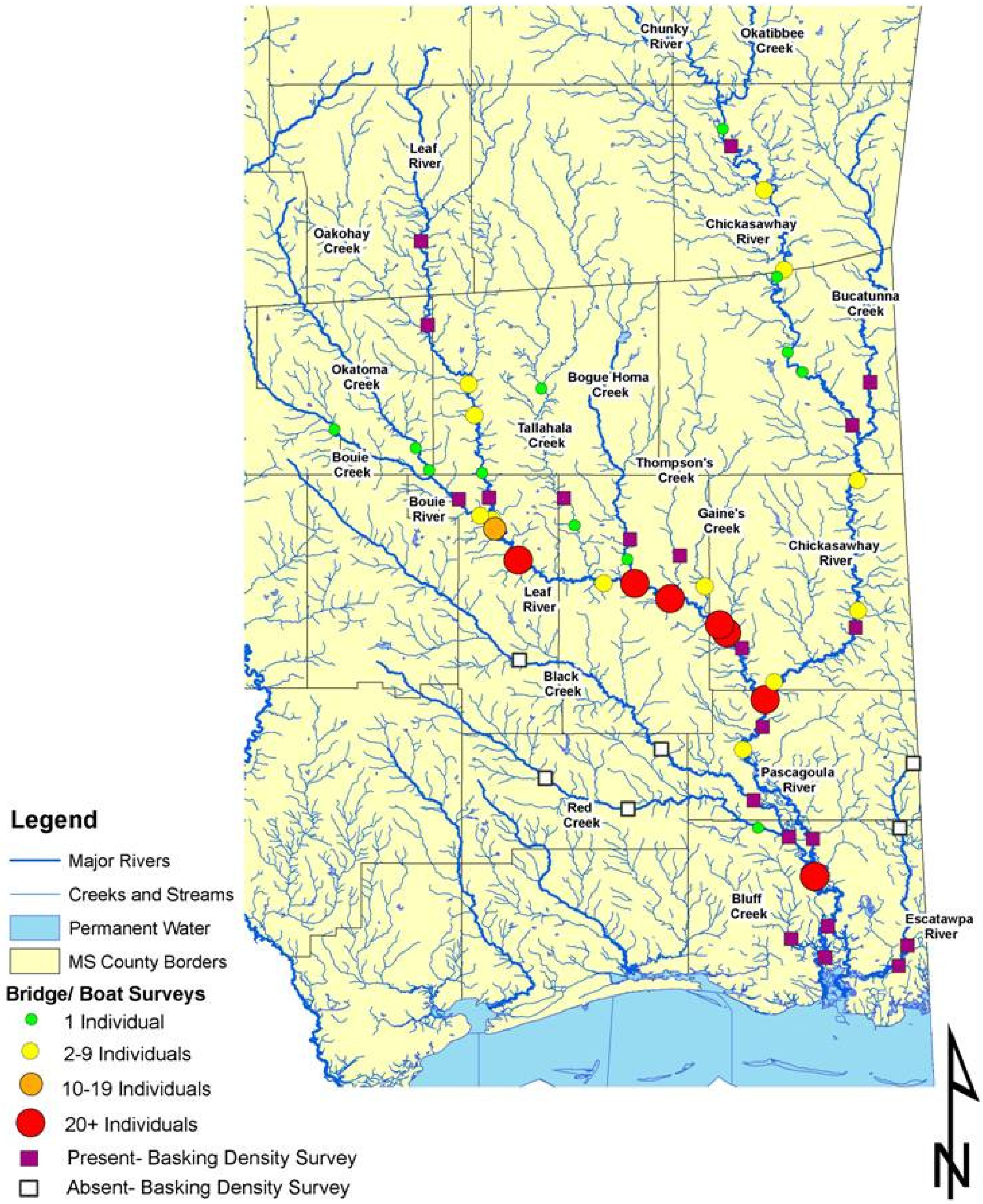


Figure 3.2. Bridge and basking density survey results for *G. flavimaculata* within the Pascagoula River system, 2006-2008.

(north 46.25 rkm; Cliburn 1971) to the vicinity of Ellisville, MS (Jones Co.). Downstream limits for *G. flavimaculata* were observed within 13.5 rkm north of the mouth of the Pascagoula River; individuals have been captured at the mouth of the Pascagoula River, near U.S. Hwy 90 (Tom Mann, pers. comm.), but these likely represent vagrant individuals.

We found that only 31 (19%) of 161 bridge crossings that were surveyed had *G. flavimaculata* present (Table 3.1). However, when *G. flavimaculata* were present at bridge crossings, multiple individuals were usually observed basking (mean of 9.83 individuals). *Graptemys flavimaculata* was the second most abundant basking turtle observed within the Pascagoula River system (29% of 1041 basking turtles observed), while *P. concinna* was the most abundant (39%). Within the Pascagoula and its larger tributaries, *G. flavimaculata* was the most abundant basking *Graptemys* (40%; Table 3.1), particularly within the Pascagoula (84 % of basking turtles) and the Leaf (63 % of basking turtles) rivers. *Graptemys flavimaculata* was the fifth most abundant basking species within smaller rivers/large creeks (3%; behind *P. concinna*, *G. gibbonsi*, *Apalone sp.*, and *Trachemys scripta*) and was absent from medium/small creeks (Table 3.1). The highest relative densities of *G. flavimaculata* were in the Pascagoula and lower Leaf rivers, between Vancleave and Hattiesburg, as McCoy and Vogt (1980) suggested (Figure 3.2). Lower densities were found in regions of the upper Leaf River, Chickasawhay River, and several medium size creeks (Figure 3.2; Table 3.1 & 3.2). Basking density surveys by boat/canoe aligned well with bridge survey results which indicated that the largest populations of *G. flavimaculata* exist from the lower Pascagoula

Table 3.1. Bridge survey results by drainage. *G.f.*= number of *G. flavimaculata* observed, *G.g.*= number of *G. gibbonsi* observed. *Mean Basking Turtles per Bridge* includes non-*Graptemys* basking species. See Fig. 3.1 and Appendix 1 for survey locations.

Drainage	No. Bridges Surveyed	*G.f.*	*G.g.*	Mean Basking Turtles per Bridge
Bouie River	2	9	1	24.0
Chickasawhay River	13	34	57	10.2
Leaf River	16	180	108	26.8
Pascagoula River	3	66	13	28.3
Total: Large and Medium Rivers	**34**	**289**	**179**	**21.2**
% of Basking Turtles Observed (721)		**40.1**	**24.8**	
Black Creek	12	-	6	2.8
Bogue Homa	6	1	10	3.3
Bouie Creek	10	1	-	1.8
Bucatunna Creek	13	-	9	2.8
Chunky Creek	4	-	7	10.3
Escatawpa River	3	-	-	0
Gaines Creek	3	2	1	0.7
Okatoma Creek	5	2	-	2.6
Red Creek	7	-	6	1.9
Tallahalla Creek	16	2	18	4.1
Tallahoma Creek	11	-	9	1.9
Thompson's Creek	2	-	2	3.5
Total: Small Rivers and Large Creeks	**92**	**8**	**68**	**2.2**
% of Basking Turtles Observed (260)		**3.1**	**26.2**	
Atkisson Creek	1	-	-	0
Big Creek (Chickasawhay Drainage)	2	-	-	0.5
Big Creek (Leaf Drainage)	1	-	-	0
Chickasawhay Creek	1	-	-	0
Hurricane Creek	1	-	-	0
Little Black Creek	1	-	-	0
Long Creek	2	-	1	0.6
Nuakfuppa Creek	1	-	-	0.3
Oakohay Creek	5	-	1	1.4
Okatibbee Creek	5	-	4	1.2
Piney Woods Creek	1	-	-	0
Sandhill Creek	1	-	-	0
Souinlovey Creek	6	-	4	2.8
Sowashee Creek	5	-	-	1.9
Tallahata Creek	1	-	-	2.0
West Tallahala Creek	1	-	-	8.0
Total: Medium and Small Creeks	**35**	**0**	**10**	**1.7**
% of Basking Turtles Observed (60)		**0.00**	**16.7**	
Number of Bridges with Species Present (%)		31 (19)	66 (42)	
Number of Bridges with Species Absent (%)		128 (81)	93 (58)	
Mean observed per bridge when Species Present		9.83300	4.38000	
Total Observed (Overall 1041)	**161**	**297**	**257**	
Percentage of Total		**0.28530**	**0.24688**	

River (vicinity of Wade/Vancleave, Jackson Co.), upstream in the Leaf River to the confluence of the Bouie River (vicinity of Hattiesburg, Forrest Co.; Table 3.2). Basking density surveys also recorded low abundances of *G. flavimaculata* within several medium-sized creeks, as was also confirmed by bridge survey.

Graptemys flavimaculata were not found in apparently suitable habitats in the middle portions of Black Creek, Red Creek, and Escatawpa rivers. Additionally, we did not observe *G. flavimaculata* within the non-flowing, lake sections of the lower Bouie River which were created by gravel mining operations, while also documenting the absence of individuals in areas impacted by gravel mining within Thompson's Creek. However, we did see individuals in historical flowing river channels between Bouie lakes and also found turtles inhabiting intact sections upstream and downstream of impacted areas in Thompson's Creek. Also, we noted few individuals in the channelized and de-snagged portion of the Leaf River (3.8 rkm) adjacent to Hattiesburg, MS (Forrest Co.) and downstream of a municipal wastewater input southeast of Hattiesburg.

Graptemys gibbonsi *Distribution and Relative Abundance.*—We found *G. gibbonsi* inhabiting all the major rivers including the Pascagoula, Chickasawhay, and Leaf rivers, as well as the historical localities of Red Creek, Black Creek, Thompson's Creek, Tallahala Creek, and the Chunky River (Cliburn 1971, McCoy and Vogt 1980, Lindeman 1998). We report previously undocumented drainage localities for *G. gibbonsi* including: Bogue Homa (Perry and Jones Co.'s), Bucatunna (Wayne, Clarke, Lauderdale Co.'s), Long (Clarke Co.), Gaine's (Perry Co.), Oakohay (Covington, Smith Co.'s), Okatibbee (Lauderdale Co.), Souinlovey (Clarke Co.), and Tallahoma creeks (Jones, Jasper Co.'s;

Table 3.2: Basking density surveys of *Graptemys* within the Pascagoula River system, including major and minor tributaries. *G.f.* F = *Graptemys flavimaculata* females, *G.f.* M = *G. flavimaculata* males, *G.f.* T = Total *G. flavimaculata*, *G.g.* F = *Graptemys gibbonsi* females, *G.g.* M = *G. gibbonsi* males, and *G.g.* T = Total *G. gibbonsi*. nt = data not taken on sex of individuals observed. * = indicates site within range of *G. flavimaculata* recovery plan. † = indicates site that meets requirements suggested by *G. flavimaculata* Recovery Plan. See Fig. 3.1 for locations of numbered survey sites.

				Turtle Basking Density (turtles per km)					
Drainage	Site	Date Surveyed	Distance Surveyed (km)	*G.f.* F	*G.f.* M	*G.f.* T	*G.g.* F	*G.g.* M	*G.g.* T
Pascagoula River Site*†	1	10/31/07	2.0	32.5	31.5	67	-	1.0	1.0
Pascagoula River*†	2	7/15/08	3.2	45.9	85.3	139.2	4.0	8.9	13.7
Pascagoula River*†	3	7/15/08	4.5	14.0	39.2	57.3	6.4	10.5	20.4
Total Pascagoula			**9.7**	**30.8**	**52**	**87.8**	**3.5**	**6.8**	**11.7**
Leaf River*†	4	7/31/08	4.2	16.1	36.5	60.0	17.3	22.9	44.5
Leaf River Site*†	5	10/15/07	3.8	8.3	14.7	23.7	3.5	4.0	7.7
Leaf River*	6	7/9/08	22.2	2.0	1.7	3.8	4.2	1.5	5.9
Leaf River*	7	7/10/07	22.9	0.1	0.2	0.3	2.5	0.6	3.3
Total Leaf			**53.1**	**6.6**	**13.3**	**22**	**6.9**	**7.3**	**15.3**
Chickasawhay River*†	8	9/24/08	3.2	5.6	16.9	24.9	10.5	12.9	27.3
Chickasawhay River*	9	7/29/08	8.0	1.6	4.5	6.4	7.1	3.9	14.9
Chickasawhay River*	10	6/16/08	5.2	1.0	2.0	3.0	4.0	5.0	10.5
Total Chickasawhay			**16.4**	**2.7**	**7.8**	**11.4**	**7.2**	**7.3**	**17.6**
Escatawpa River	11	6/28/06	9.7	nt	nt	4.2	-	-	-
Escatawpa River	12	10/20/08	4.0	-	-	-	-	-	-
Escatawpa River	13	10/2/08	10.6	-	-	-	-	-	-
Total Escatawpa			**24.3**	**nt**	**nt**	**1.4**	**-**	**-**	**-**
Black Creek	14	7/7/08	8.0	1.3	2.3	4.2	1.3	1.6	5.2
Black Creek	15	6/6/08	15.9	-	-	-	2.3	0.7	3.7
Black Creek	16	7/11/07	8.1	-	-	-	0.6	1.0	3.4
Red Creek	17	6/23/08	22.4	-	-	-	1.5	0.5	2.5
Red Creek	18	8/7/07	15.7	-	-	-	0.3	0.3	0.9
Bluff Creek	19	9/16/06	6.5	0.9	0.2	1.1	-	-	-
Tallahala Creek	20	9/23/08	16.9	1.7	2.6	4.3	2.9	2.6	7.5
Bogue Homa Creek	21	6/6/07	17.1	0.3	1.1	1.7	4.1	0.5	4.9
Thompson's Creek	22	9/25/08	10.1	0.8	0.6	1.4	1.3	1.0	2.9
Bucatunna Creek	23	7/13/07	15.3	-	0.2	0.2	1.9	1.4	5.3
Total Creeks			**136**	**0.5**	**0.7**	**1.3**	**1.6**	**1.0**	**3.6**
Total			**239.5**	**8.1**	**14.8**	**24.8**	**3.8**	**4.5**	**9.6**

Figure 3.3). We did not observe *Graptemys gibbonsi* during the basking density survey in the Escatawpa River, but we observed four individuals basking in the vicinity of Goode's Mill Lake (Jackson Co.) after working multiple days in that system; this was unexpected considering that Mount (1975) did not record this species in the Alabama portion of the Escatawpa River. We suspect that this population is also geographically disjunct from the Pascagoula River for the same reasons as *G. flavimaculata*. We also documented range extensions for *G. gibbonsi* within the Leaf River (north approx 46 rkm; Cliburn 1971), Red Creek (northwest approx 77 rkm; Cliburn 1971), and Tallahala Creek (north approx. 67 rkm; Cliburn 1971). The downstream limits for *G. gibbonsi* appear to be approximately 20 rkm north of the Pascagoula River mouth.

We found *Graptemys gibbonsi* at 66 of 161 surveyed bridge crossings (42 %, Table 3.1). *G. gibbonsi* was less abundant than *G. flavimaculata* at bridge crossings, as there was on average only 4.38 individuals of this species basking per bridge which is 55% less than the mean number of *G. flavimaculata* (9.83 individuals) present. Of 1041 total turtles observed during bridge surveys, *G. gibbonsi* comprised 25 percent (257 turtles) of the basking turtles seen, the third most common basking species behind *P. concinna* and *G. flavimaculata*. *Graptemys gibbonsi* was the dominant basking *Graptemys* only within the Chickasawhay River (63 percent). However, the Chickasawhay River had fewer total basking turtles observed per bridge (10.2) compared to the Pascagoula (28.3) or Leaf rivers (26.8; Table 3.1). Within the Pascagoula and its larger tributaries, *G. gibbonsi* was the third most abundant basking turtle (25%), behind *G. flavimaculata* and *P. concinna*. *G. gibbonsi* was the second most abundant basking species within smaller rivers/large creeks (26%; behind *P. concinna*) and was the third

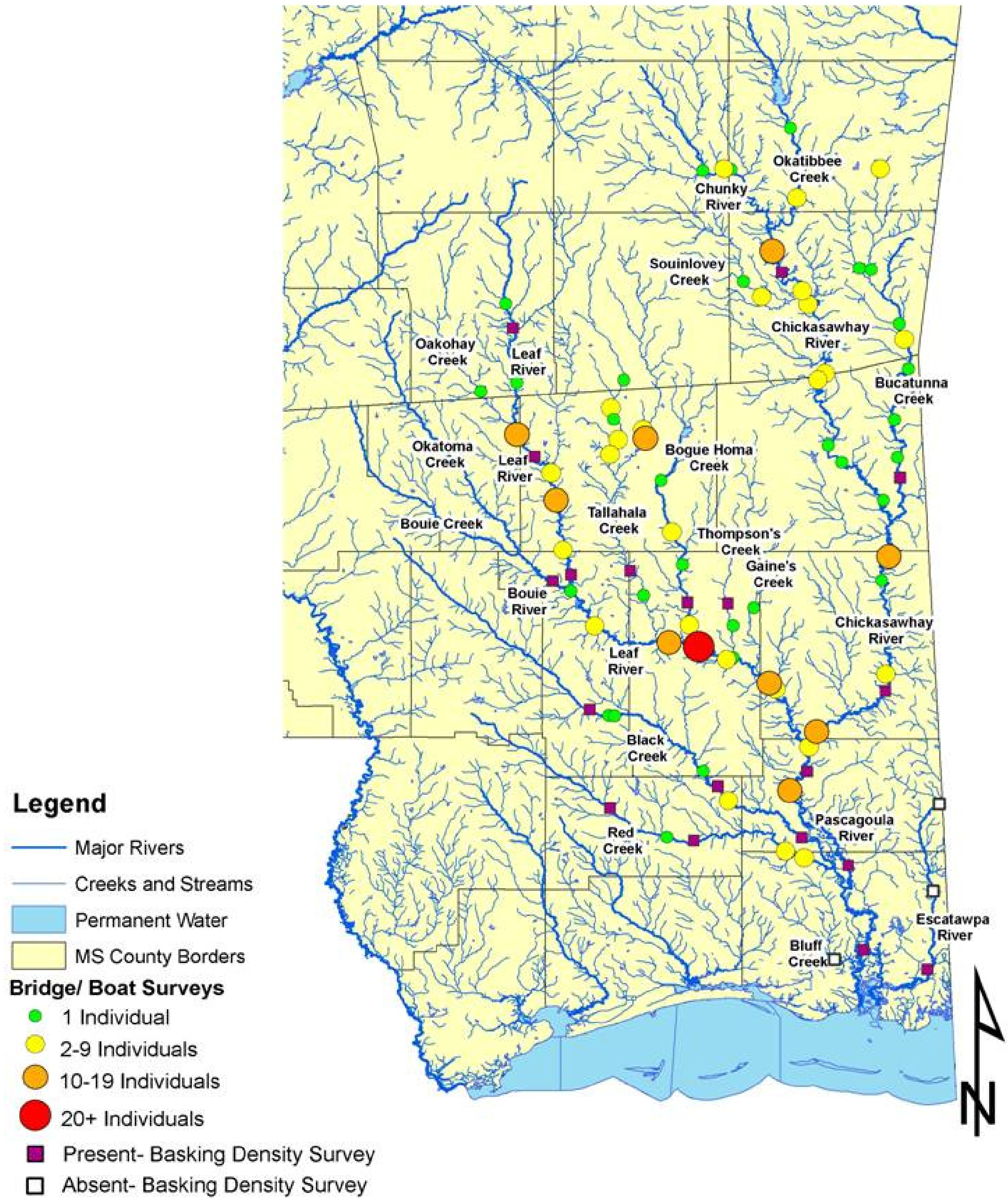


Figure 3.3. Bridge and basking density survey results for *G. gibbonsi* within the Pascagoula River system, 2006-2008.

most abundant basking turtle in medium/small creeks (17%; behind *P. concinna* and *T. scripta*).

The relative density of *G. gibbonsi* within the Pascagoula River system exhibits no clear pattern (Figure 3.3). This species appears to be locally abundant in the upper reaches of the Pascagoula River and sections of the Leaf and Chickasawhay rivers, but between these areas of high abundance, very few individuals were noted. This is in contrast to the relatively consistent numbers of *G. flavimaculata* observed in the Leaf and Pascagoula rivers during the same surveys. Basking density surveys closely mirrored bridge surveys and also indicated that populations of *G. gibbonsi* within the Pascagoula River system were highly variable (Table 3.2), appearing to be locally abundant within the upper Pascagoula River and lower Leaf River, and in areas of the Chickasawhay River where they were more abundant than *G. flavimaculata*. Bridge surveys did not document *G. gibbonsi* within the middle portion of Tallahala Creek and its presence there was only confirmed during a basking density survey by canoe.

We did not observe individuals during bridge surveys in either Okatoma or Bouie creeks where they had been previously documented. A boat survey of the lower reaches of the Bouie River noted one individual, but this stretch of river has been severely impacted due to gravel mining. Individuals were also conspicuously absent from gravel mining impacted reaches within Thompson's Creek. Furthermore, populations within Tallahala and Tallahoma creeks have higher density above the town of Laurel (Jones Co.) than downstream in portions of Tallahala Creek, which was historically grossly polluted due to point sources in the vicinity of Laurel (Cliburn, pers. comm.; Cagle 1954; Figure 3.3). Lastly, we documented very few *G. gibbonsi* in the lower reaches of the Pascagoula

River and no individuals were spotted within Bluff Creek which has no previously documented records for *G. gibbonsi*.

Population estimates and species ratios.—We conducted population estimates for *G. flavimaculata* at 3 sites in 2005 and 2 sites in 2006-2008. In 2005, the Chickasawhay River site (Greene County) had an estimated 232 *G. flavimaculata* (95% CI: 127-419) in 2.4 river km (93 per rkm; Table 3.3). From 2005-2008, estimates for the Leaf River site (Forrest County) ranged from 257-451 (95%CI: 145-1143) turtles in 3.75 rkm (80-120 per rkm). The lower Pascagoula River site (Jackson County) was also surveyed from 2005-2008 and population estimates ranged from 562-1203 (95% CI: 381-2339) turtles in 2.0 rkm (281-602 per r/km). We could only complete two population estimates for *G. gibbonsi*, both at the Leaf River site. This was primarily due to the inability to catch and paint-mark enough individuals for a reliable population estimate at other sites. The Leaf

Table 3.3. NOREMARK population estimates for *G. flavimaculata* (*G.f.*) and *G. gibbonsi* (*G.g.*) from 2005 – 2008 within the Pascagoula River system, Mississippi, USA.

Species	**Year**	**Site**	**Distance Surveyed (river km)**	**Population Estimate**	**95 % CI**	**Estimated Turtles/km**
G.f.	2005	Chickasawhay	2.4	223	127-419	93
G.f.	2005	Leaf	3.2	257	146-482	80
G.f.	2006	Leaf	3.75	451	202-1144	120
G.f.	2007	Leaf	3.75	361	222-726	96
G.f.	2008	Leaf	3.75	335	245-501	89
G.f.	2005	Pascagoula	2.0	1203	732-2339	602
G.f.	2006	Pascagoula	2.0	641	433-989	321
G.f.	2007	Pascagoula	2.0	562	381-946	281
G.f.	2008	Pascagoula	2.0	583	402-953	292
G.g.	2007	Leaf	3.75	129	102-179	34
G.g.	2008	Leaf	3.75	166	109-293	44

River site had an estimated 129-166 (95% CI: 102-293) turtles in 3.75 rkm (34-44 per rkm; Table 3.3).

Trapping results also indicated that *G. flavimaculata* outnumbered *G. gibbonsi* at all three sites surveyed (Table 3.4). The Chickasawhay River site had the lowest *G. flavimaculata* to *G. gibbonsi* ratio (1.75:1), whereas during 4 years of trapping at the Lower Pascagoula River site, only 19 *G. gibbonsi* were trapped compared to 479 *G. flavimaculata* (25.2:1). The latter site was also juvenile-dominated as 16 out of 19 *G. gibbonsi* captured were of juvenile size.

Table 3.4. Trapping results for 2005 - 2008 at three sites within the Pascagoula River system for *Graptemys* species. Species listed are *Graptemys flavimaculata* (*G.f.)* and *Graptemys gibbonsi* (*G.g.*).

Site/ River	Year	Species	Total Captured (# of F, M, Juv)	Capture Ratio (*G.f.* to *G.g.*)
Leakesville	2005	*G.f.*	73 (30, 35, 8)	
Chickasawhay		*G.g.*	45 (4, 31, 10)	1.62 : 1
Leakesville	2006	*G.f.*	50 (12, 26, 12)	
Chickasawhay		*G.g.*	25 (3, 19, 3)	2.00 : 1
Hattiesburg	2005	*G.f.*	65 (18, 42, 5)	
Leaf		*G.g.*	31 (13, 10, 8)	2.10 : 1
Hattiesburg	2006	*G.f.*	73 (25, 44, 4)	
Leaf		*G.g.*	20 (9, 9, 2)	3.65 : 1
Hattiesburg	2007	*G.f.*	92 (32, 48, 12)	
Leaf		*G.g.*	27 (9, 13, 5)	3.41 : 1
Hattiesburg	2008	*G.f.*	96 (24, 51, 21)	
Leaf		*G.g.*	31 (7, 18, 6)	3.10: 1
Vancleave	2005	*G.f.*	102 (34, 65, 3)	
Pascagoula		*G.g.*	1 (0, 0, 1)	102 : 1
Vancleave	2006	*G.f.*	127 (51, 77, 4)	
Pascagoula		*G.g.*	2 (1, 0, 1)	63.5 : 1
Vancleave	2007	*G.f.*	119 (45, 60, 14)	
Pascagoula		*G.g.*	8 (0, 2, 6)	14.88 : 1
Vancleave	2008	*G.f.*	131 (55, 48, 27)	
Pascagoula		*G.g.*	8 (0,0,8)	16.38 : 1

DISCUSSION

Graptemys flavimaculata *status.*— The presence of *G. flavimaculata* in all previously recorded localities, as well as in new ones, suggests that *G. flavimaculata* has not undergone a range contraction as was suggested by Lindeman (1999). Lovich (in Ernst and Lovich 2009) indicated that *G. flavimaculata* was noticeably absent from reaches below a known paper mill effluent within the Leaf River (New Augusta, Perry Co.), but our study found this reach to have one of the highest numbers of *G. flavimaculata* and *G. gibbonsi* observed by bridge and by basking density surveys (site number 4). This could be due to improved water quality from a cleaner paper mill effluent in the intervening years.

Further, our population estimates and basking density surveys at all localities were generally higher than previously reported (U.S. Fish and Wildlife Service 1991; Lindeman 1998). Recovery requirements for *G. flavimaculata* indicate that populations should meet or exceed 44 turtles per rkm in the Pascagoula and 22 turtles per rkm in the Leaf and Chickasawhay rivers (U.S. Fish and Wildlife Service 1993). Population estimates exceeded the USFWS recovery requirements at all 3 mark-resight localities. The population estimates at the Leaf River site and Chickasawhay River site were considerably higher than what was found during the early 1990's when there were approximately 4 basking *G. flavimaculata* per river mile in 40 miles of the Leaf River and 20 miles of the Chickasawhay (U.S. Fish and Wildlife Service 1991). However, USFWS surveys were conducted by moving boats which have been noted to startle turtles even before they come into the turtle's view (W. Selman, pers. obs). Therefore, these surveys were likely a very low estimate of population densities for *G.*

flavimaculata, especially if weather conditions were not conducive to basking (i.e., cloudiness, summer months). Additionally, basking density surveys met or exceeded recovery requirements at 6 of 10 sites within the Pascagoula River (3 of 3), Leaf River (2 of 4), and Chickasawhay River (1 of 3; Table 3.2). However, within the Pascagoula River system and the lower 129 rkm of the Leaf and Chickasawhay rivers (specified habitat to be protected in recovery plan), 6 of 6 surveys met or exceeded recovery plan objectives (sites 1, 2, 3, 4, 5, 8); the other survey reaches within the upper Leaf and Chicksawhay rivers that did not meet recovery requirements (sites 6, 7, 9, 10) will likely never meet these requirements due to smaller river characteristics and patchiness of optimal habitat for this species.

Graptemys flavimaculata was also found in much smaller drainages (15-20 m wide) than previously described for this species. Within these smaller creeks, it is uncertain how much area is occupied by turtles since there are river stretches of apparently suitable habitat (open canopy, snags, moderate flow, sandbars) separated by kilometers of apparent marginal/unsuitable habitat (closed canopy, few snags, high flow with rapids, no sandbars). It was previously uncertain if *G. flavimaculata* used these smaller rivers/creeks for year-round habitat or if they are seasonal refuges. But if these smaller rivers/creeks are used only seasonally, one might expect the absence of adult females and hatchlings/juveniles, due to the more suitable habitat of larger rivers that have higher quality, large nesting sandbars. However, adult females were encountered in the upper Leaf River and Bogue Homa, Gaine's, Okatoma, and Tallahala creeks; hatchlings/juveniles were found within the upper Leaf River and Black, Bogue Homa, Buckatunna, and Tallahala creeks. Therefore, these smaller size drainages (<25 m wide) appear to offer year-round habitat, including nesting habitat and suitable year-round food resources.

It is uncertain why *G. flavimaculata* does not inhabit middle and upper portions of the Escatawpa River, Black Creek, and Red creek. These areas appear to offer similar and/or better habitats than other localities where they were observed (i.e., Okatoma and Bucatunna creeks). The only apparent dispersal barrier between these drainages and the Pascagoula River appears to be when they encounter the Pascagoula River floodplain, where the creeks become narrow with few suitable sandbars and a closed tree canopy over the creek/river. However, individuals were noted above this area within both Black and Red creeks, and therefore, appear to have overcome this barrier. Further, these stretches are considered 'blackwater' habitat (clear, tannin stained waters), which likely contains fewer nutrients and may historically lack primary prey items; this, however, does not explain the presence of *G. gibbonsi* in these portions of Red and Black creeks. The absence of *G. flavimaculata* and *G. gibbonsi* from middle and upper portions of the Escatawpa River are likely due to the absence of many freshwater mollusks from this drainage (Williams et al. 2008). Regardless, these areas should be surveyed in the future to determine if expansion is occurring within these creeks, particularly since these river/creek systems could harbor the exotic Asian Clam (*Corbicula fluminea*) in the future, a food item of both *Graptemys* species (Ennen et al. 2007, W. Selman, unpubl. data).

The decline of the largest population of *G. flavimaculata* in the lower Pascagoula River is disconcerting. This population decreased significantly (by approximately 47%) after 2005, presumably due to the negative impacts from Hurricane Katrina (Selman and

Qualls 2008) and did not rebound in the three following years. However, during 2007 and 2008 the percentage of juveniles captured increased dramatically (41 of 250; 16.4%) in comparison to 2005 and 2006 (7 of 229; 3.1%). However, since we did not focus on the nesting of *G. flavimaculata* during this study, we are unsure of the exact mechanism that led to increased juvenile frequency in the lower Pascagoula River following Hurricane Katrina. Some possibilities may include: three consecutive dry years following Hurricane Katrina which might have increased nest/hatching success, lower nest mortality due to flooding (Horne et al. 2003) during these dry years, lower numbers of nest predators due to predator mortality associated with the Hurricane Katrina storm surge/flooding, and/or better riverine conditions; all of these possibilities warrant future study. This population in the future, however, is highly susceptible to declines due to a multitude of threats that do not exist in other populations, of which many are due to the dramatic human population growth in southeast Mississippi (i.e., excessive recreational boating traffic, likely salinity increases due to municipal/industrial freshwater withdrawals, human collection).

G. gibbonsi *status.—Graptemys gibbonsi* was found in many localities where it was previously documented, as well as some new drainages and range extensions. Previous surveys may have overlooked many of these ‘new’ drainage localities because of their small sizes, so it is unknown to what extent *G. gibbonsi* historically occurred within these drainages. This species was found to be in much smaller drainages (i.e., upper Bucatunna and Long creeks) than was *G. flavimaculata*. However, these drainages likely contain smaller populations compared to the larger streams that *G. gibbonsi* inhabits.

The absence of *G. gibbonsi* from Bouie and Okatoma creeks, where it occurred historically, is clear. We suspect that its absence is likely due to periods of historically poor water quality within these drainages. J.W. Cliburn (pers. comm.) noted that these creeks were highly polluted during the 1970's and therefore, this species may have been locally extirpated in these drainages. This would likely be due to the loss of main prey items, which are primarily mollusks for females (Ennen et al. 2007, Lindeman and Sharkey 2001). These drainages currently appear to be suitable habitat for *G. gibbonsi* and both contained *G. flavimaculata*, but we suspect that *G. flavimaculata* may have persisted in these drainages because of a more generalized diet (Seigel and Brauman. 1994). A similar situation was noted within the Pearl River near Columbia, downstream of a pulp mill, where *Graptemys oculifera* (Ringed sawback) populations remained stable over 17 years, while *G. gibbonsi* declined over the same time period (W. Selman and R.L. Jones, unpub. data). *Graptemys gibbonsi*, therefore, could be more prone to declines due to the loss of mollusk populations which have been documented in Mississippi (Jones et al. 2005).

G. gibbonsi population levels could not be determined in the Chickasawhay River or lower Pascagoula River due to the difficulty in catching enough individuals for a mark-resight survey. Only two population estimates were completed at the Leaf River site and the population was found to be 2-3.5 times lower than *G. flavimaculata* population levels in the same river stretch (Table 3.1). However, if we extrapolate trapping data from our other two sites that lack population estimates and compare them to our Leaf River population estimate, *G. gibbonsi* likely occurs at much lower levels in the lower Pascagoula River but at similar levels in the Chickasawhay River.

Thus, bridge surveys, basking density surveys, and trapping data from the Pascagoula River system indicate that *G. gibbonsi* occurs in much lower abundance throughout the drainage in comparison to *G. flavimaculata*. In contrast, Tinkle (1958) found these species in nearly similar abundance within the Pascagoula River (21 *G. flavimaculata* to 15 *G. gibbonsi*). In addition, populations of the federally Threatened *G. oculifera* were surveyed within West Pearl River and Bogue Chitto River (Pearl River system) of Louisiana and *G. gibbonsi* populations were in lower numbers in both surveys in comparison to *G. oculifera* (Dickerson and Reine 1996, Shively 1999). In addition, over the last 17 years in the lower Pearl River (vicinity of Columbia, MS), *G. gibbonsi* populations have dropped from 46 percent of the captured *Graptemys* in 1990 to 17% in 2006; over the same time period, *Graptemys oculifera* populations remained stable (Jones and Selman, unpubl. data). Therefore, in summary, many factors could complicate management decisions for *G. gibbonsi* including: smaller population sizes within the Pascagoula and Pearl rivers than either of the Threatened species (*G. flavimaculata* and *G. oculifera*); it has a wider distribution than *G. flavimaculata*; it is a dietary specialist (Ennen et al. 2007, Lindeman and Sharkey 2001); and there are genetic differences among the two major drainages inhabited (Ennen et al., unpubl. data). Therefore, we concur with Lindeman's (1998, 1999) recommendation that *G. gibbonsi* should be federally listed as Threatened and be designated as Endangered (EN) by the IUCN. We also suggest state endangered status for *G. gibbonsi* in Mississippi and Louisiana.

Drainage Comparisons.—There appear to be major differences in turtle densities between the two major rivers of the Pascagoula River system, the Leaf and Chickasawhay rivers. This is supported by our bridge and basking density surveys that found similar densities of both *Graptemys* species in the Pascagoula and lower Leaf rivers, but much lower densities in the Chickasawhay River (Tables 3.1 & 3.2). Additionally, our population estimates from the Chickasawhay and Leaf River sites are similar (Table 3.3), but the Leaf River site is approximately 121 rkm upstream from the origin of the Pascagoula River, whereas the Chickasawhay River site is only 43.8 rkm; this indicates that good populations of *G. flavimaculata* extend much further up the Leaf River in comparison to the Chickasawhay.

The Leaf River appears to be more similar to the Pascagoula River in that they both have large meander sections with associated nesting sandbars and large amounts of deadwood in cutbank river sections. The Chickasawhay River, on the other hand, is a more north-to-south river and it cuts across rather different geological formations (Bicker 1969) than the Leaf, in particular, rocky strata in the headwaters. In addition, the river level fluctuates quite rapidly in the Chickasawhay relative to the Leaf River, likely due to a more restricted channel in the headwaters of the Chickasawhay River. This could lead to swifter river currents, shadier river sections, fewer nesting beaches, and overall lower nutrients within the Chickasawhay River system, which could contribute to the overall lower density of turtles within this river. Support for this conclusion also comes from a study with the Alabama Shad (*Alosa alabamae*) which found a lower growth rate and body condition of juvenile shad from the Chickasawhay River relative to the Leaf River (Mickle et al. 2009); this fish species feeds partly on aquatic insects, a *Graptemys* prey item.

Conservation Recommendations.—We believe in order to maintain critical habitat for both species, there should be continued riparian land acquisition along the major tributaries of the Pascagoula (Leaf and Chickasawhay rivers) that harbor *Graptemys* populations. Additionally, efforts should continue to curtail activities that aim to alter the Pascagoula River and its tributaries, including the pending Federal Strategic Petroleum Reserve project. This project plans to withdraw water (~50 million gallons/day) from the Pascagoula River to dissolve a salt cavern for oil storage, as well as carrying (by pipeline) the salt brine by-product to dispose in the Gulf of Mexico. This project has the potential to cumulatively impact the river system by potentially lowering water levels, by saltwater intrusion in coastal areas due to lower river discharge, through a break in a salt brine-carrying pipeline causing hypersaline brine discharge into freshwater systems, or by salt brine influx to the river following brine disposal into the Gulf of Mexico. This project and others like it will not do anything positive for this river system, but they have the possibility to negatively impact the relatively pristine aquatic communities of this river system.

Appropriate conservation measures for *Graptemys* species within the Pascagoula River system should include protecting and improving habitat suitability of occupied rivers/creeks; discouraging habitat alteration (i.e. de-snagging projects, impoundments, channelization, poorly planned riparian land developments); controlling invasive species; and law enforcement to limit illegal collection, shooting of turtles, and ATV use on nesting sandbars. We also support any efforts to offer conservation incentive programs to riparian landowners throughout the Pascagoula River system, as well as policies to protect riparian zones. These should decrease the impact of sedimentation on rivers which decreases prey availability and will greatly improve *Graptemys* habitat.

Conclusions and Future Survey Recommendations.—Our data indicate that there has not been an observable range contraction for *G. flavimaculata* since 1970, but possibly a range expansion; it is uncertain if this is a 'real' expansion or an artifact of the more exhaustive survey effort of this study. Additionally, it appears that *G. flavimaculata* populations appear more robust than recorded during previous surveys (U.S. Fish and Wildlife Service 1991; Lindeman 1998, 1999). However, the largest population of *G. flavimaculata* within the lower Pascagoula was severely impacted during our study following Hurricane Katrina and many threats continue to exist due to the dramatic human population increase of coastal Mississippi counties. Our data also provides additional support to Lindeman's (1999) recommendation to federally list and upgrade the IUCN status of *G. gibbonsi*. With the little historical information that is available, it is probable that *G. gibbonsi* is declining, while already having lower rangewide population density than either of the two federally Threatened *Graptemys* species (*G. flavimaculata* or *G. oculifera*).

Lastly, our study indicates that even though the distribution of a species has been well studied (i.e. *G. flavimaculata*), additional new populations and/or new drainage localities may exist. Several localities that were deemed too narrow or shallow for *Graptemys* by McCoy and Vogt (1980) were found by our surveys to contain *Graptemys* populations. Additionally, four localities for *G. flavimaculata* were thought to not have populations from our bridge survey results (Bucatunna Creek, Thompson's Creek, Lower Black Creek, and Upper Leaf River) and were only later confirmed after basking density surveys by canoe/boat were completed in the same stretch of river. Even more striking was that *G. gibbonsi* populations were not confirmed by either bridge survey or basking density survey in the lower Escatawpa, but only after spending multiple days in the area. Therefore, we suggest that more rigorous range-wide studies should be initiated for other imperiled southeastern riverine turtle species using multiple methods as this study has to establish baseline distributional and population status data. Surveys should focus in core habitats and existing localities, as well as those areas that have been unsurveyed and/or are believed to be unsuitable habitat. Thereafter, these studies should then be able to provide quality information to individuals making conservation decisions for species or aquatic ecosystems.

CHAPTER IV

BASKING ECOLOGY OF THE YELLOW-BLOTCHED SAWBACK (*GRAPTEMYS FLAVIMACULATA*)

Abstract.—The role of basking in chelonians is an understudied behavior, especially considering how conspicuous it is in some species. Of the past studies conducted on turtle basking behavior, researchers have suggested many physiological roles that basking likely fulfills in turtles. We documented basking behavior of an imperiled *Graptemys* species of the Southeastern United States, the Yellow-blotched sawback (*Graptemys flavimaculata*), on the Leaf River, a tributary of the Pascagoula River in southeastern Mississippi. We used binoculars and/or a spotting scope to determine *G. flavimaculata* individual and population level basking behaviors throughout the main active months (April- October) and across the daily activity period; we also describe a new method to determine population basking percentage that may be useful for future aquatic turtle surveys. We found distinct differences in individual and population level basking behavior across months, sexes, and the daily activity period. In addition, we also documented differences among sexes in basking structures used, but found little correlation with population level basking related to several environmental temperature variables. Our results suggest that there are still many questions to be answered and that future studies should continue to explore this interesting behavior, particularly in the area of basking and individual physiological needs throughout the year.

INTRODUCTION

Basking activity of aquatic Emydid turtles is one of the most conspicuous daily behaviors exhibited by this group, making it an easily studied topic for researchers. Early studies assumed that the primary physiological role of basking in turtles was to regulate body temperatures, as well as condition the skin and shell (Cagle 1950, Boyer 1965, Auth 1975). Moll and Legler (1971) hypothesized that basking increases metabolism and digestion rates. Others suggested that basking aids in vitamin K synthesis (Pritchard and Greenhood 1968), increases follicular/egg production in female turtles during the nesting season (Hammond et al. 1988, Krawchuck and Brooks 1998), allows turtles to rest (Boyer 1965, Waters 1974), fights infection by "behavioral fever" (Monagas and Gatten 1983), and rids turtles of ectoparasites (Cagle 1950, Neill and Allen 1954, Vogt 1979, Selman et al. 2008, Selman and Qualls 2009a). In summation, Moll and Legler (1971) stated that basking is likely initiated by a single impulse, which is either triggered by an external factor or internal physiological need, while other secondary benefits are thereafter gained.

Members of the genus *Graptemys* are highly aquatic, freshwater turtles with many species endemic to single river drainages of the southeastern United States (Ernst and Lovich 2009). Turtles within this genus often occur in large basking aggregations, usually on emergent deadwood snags (e.g. fallen trees from river banks). Sometimes turtles are stacked upon one another (Floyd 1973, W. Selman per. obs.) or turtles may bask up to several meters above the water surface (Floyd 1973, Vogt 1980); both of these behaviors likely function to enhance the detection of predators. Even though *Graptemys* are habitual baskers, there are relatively few recent in-depth studies of this habit of species within the *Graptemys* genus (*G. barbouri*- Sanderson 1974; *G. ernsti*- Shealy 1976; *G. geographica*- Flaherty and Bider 1984, Ecksdine 1985, Bulté and Blouin-Demers, 2009; *G. nigrinoda*- Waters 1974; *G. pseudogeographica*/ *G. oauchitensis*- Coleman and Gutberlet 2008). Although many *Graptemys* species are commonly observed within river systems of the southeastern United States, seven of the twelve species within the genus are considered imperiled (G2) or vulnerable (G3) due to their restricted distributions or endemism to single river systems (http://www.natureserve.org/explorer, accessed 31 August 2009). Furthermore, threats to *Graptemys* species include human modifications/alterations of river systems (impoundments, de-snagging operations [removal of deadwood], excess sedimentation, poor water quality), overcollection for the pet trade, fisherman bycatch, exotic/invasive species, subsidized predators, and the shooting of basking turtles by humans as target practice (Buhlmann and Gibbons 1997; Lovich et al. 2009; Jones and Selman, 2009, Selman and Jones, 2010).

The Yellow-blotched sawback (*Graptemys flavimaculata*) is an imperiled riverine turtle of the Pascagoula River system of southeastern Mississippi, USA (Selman and Jones, 2010). *Graptemys flavimaculata* was listed as Federally Threatened (U.S. Fish and Wildlife Service 1991) following observed declines in the 1980's. Following Federal listing, several studies were initiated to determine the population status (Jones 1994), home range and seasonal movements (Jones 1996), habitat associations (Lindeman 1998, 1999), reproduction/nesting ecology (Horne et al. 2003), seasonal hormone cycles (Shelby et al. 2000, Shelby and Mendonça 2001), and nesting/basking behavior in relation to human disturbance (Moore and Seigel 2006). Most of these studies were conducted in the lower Pascagoula River (Jackson County, MS) where the most robust population of *G. flavimaculata* exists (Selman and Qualls 2009b). Within the range of *G. flavimaculata*, the lower Pascagoula River population is considered one of the most threatened (Selman and Jones, 2010), primarily due to excessive nest predation by human-subsidized predators (Horne et al. 2003); recreational boating disturbances on basking behavior, nesting, and direct mortality (Moore and Seigel 2006); and the negative impacts following Hurricane Katrina (Selman and Qualls 2008).

Moore and Seigel (2006) found that many turtles within the lower Pascagoula River were disturbed by human recreational activity while basking and nesting. However, due to the magnitude of recreational activity observed during their study, it is unlikely that this population exhibited basking patterns similar to other upstream, minimally disturbed populations. Additionally, outside of the lower Pascagoula River population, little is known of the behavioral ecology of *G. flavimaculata* from smaller river systems, away from large amounts of human activity. Therefore, our study examined basking in this species by studying individual and population level basking behaviors in relation to sex, time of day, season, basking substrate type, and environmental temperatures at a site that had little human disturbance.

MATERIALS AND METHODS

Basking Observation Methods.—The study site was a relatively undisturbed locality on the Leaf River (Forrest County, Mississippi, USA). This site was selected because it was free of excessive human recreational activity that could alter turtle basking behavior. Even though two boat ramps, one located 3.8 river kilometers (rkm) downstream of the site and a second 12.7 rkm upstream of the site, are nearby, low water levels during most of the year limited accessibility by motorized boats. This stretch of the Leaf River is a medium-sized river (approx. 30 m wide) with alternating sandbar/cutbank sections. Abundant submergent/emergent deadwood and a sandy/gravel substrate are characteristic of this river stretch. This study site was also selected since previous studies documented sufficient numbers of *G. flavimaculata* in this area (Selman and Qualls 2009b).

Observations of turtle basking behavior were conducted during the months of June 2007-October 2007 and April-May 2008. Basking behavior was studied on mostly to completely sunny days with the observer located in a concealed position on a sandbar opposite of a cutbank section where turtle basking structures (i.e. emergent deadwood) were abundant. Observations were conducted with binoculars and/or a 60mm, 15 – 45 power spotting scope with tripod. Daily observations usually lasted 4 hours, but did not exceed 10 consecutive hours in a single day. Observations were attempted each month on at least two days (one weekend and one weekday) and were made during prime basking hours (i.e. 0700 hrs-1900 hrs during the summer); weather conditions did not always permit complete daily observations each month.

During observations, basking behavior was documented using two methods: individual turtle basking observations and hourly basking frequency counts. The first method was used to document individual turtle basking duration, whereas the second method was used to determine daily population level basking frequency. In the first method, when an individual *G. flavimaculata* was spotted emerging onto a basking structure, the sex of the turtle (individuals of undetermined sex and hatchlings were considered to be juveniles), time of emergence, and basking structure type were recorded.

When the individual terminated basking, the time of submergence and total basking time was recorded. When turtles were startled into the water prematurely, the apparent reason for submergence, either natural or human disturbance, was also noted. Basking structure types were categorized similar to that of Lindeman (1999), where basking structures were considered logs, branches, tree crowns, or tangles based on their size and structural complexity. However, we categorized stumps as branches or logs depending on their sizes. We could not confirm the individual identity of turtles when basking (unless a turtle had unique carapace markings) and therefore, could usually not determine the amount of time during the day that a single turtle basked.

The second method, hourly basking frequency counts, was also conducted during these observations by counting the number of basking *G. flavimaculata* within a measured, predetermined stretch of viewable river at the beginning of each hour. The sex of individuals was recorded when possible, as well as the number of other turtle species observed basking. Other basking turtle species observed included: *Apalone mutica* (Smooth Softshell), *Graptemys gibbonsi* (Pascagoula Map turtle), *Pseudemys concinna* (River Cooter), *Sternotherus carinatus* (Razorback Musk turtle), and *Trachemys scripta* (Pond Slider).

Three environmental temperatures were measured at the study site: ambient air temperature at a shaded terrestrial site, water temperature, and the temperature of a sunlit basking log. We used HOBO® Water Temp Pro v2 and Pro v2 Temperature/ External temperature data loggers (Onset Computer Corporation) to collect temperature data every five minutes at the site; water and log temperature loggers were attached to basking logs with nails and string. We collected the temperature data from the loggers with a HOBO®

Waterproof Shuttle (Onset Computer Corporation) and used HOBOware Pro data logging software (Onset Computer Corporation) to organize temperature data. We thereafter associated these temperature readings, along with three other temperature variables (1. air/water temperature difference, 2. log/air temperature difference, 3. log/water temperature difference), with each hourly basking frequency count. Temperature data were not collected during June 2007 and log temperatures could not be collected in April and May, 2008, because loggers were lost during high water levels. For the latter period, log temperature data from another site (Pascagoula River, Jackson County, MS; approximately 115 km southeast) was substituted, with the presumption that there was little difference in log temperatures across the two sites for that time period. Additionally, since water temperature loggers had to be attached to a log below the water surface and because there were naturally fluctuating river levels, water temperatures could not be collected at the same depth for every reading.

Statistical Analysis of Individual Basking Behavior.—Many turtles basked for a short period of time and few basked for longer periods of time, resulting in a Poisson distribution of individual basking observations, so we log-transformed our data to meet parametric assumptions. We analyzed yearly individual basking duration differences by sex (M, F, or Juvenile) using a one factor ANOVA; if differences were found ($p<0.05$), we used a Tukey-Kramer post hoc test to determine if the differences among sexes were significant. We also used two-factor ANOVAs to determine the effects of sex and month (April-October) on basking duration and also to determine the effects of sex and time of emergence (09:00-17:00 hrs) on basking durations of turtles. For the time of emergence, we included all turtles that emerged within a specific hour (e.g., 11:00 am to 11:59 am) were recorded as emerging at the beginning of that hour (ex. 11:00) to simplify our data analysis. Lastly, we used a chi-squared contingency table analysis to determine if the sexes used different basking structures equally.

Statistical Analysis of Population Level Basking Behavior.—To determine population level basking behavior, we calculated the percentage of the population that was basking during each hourly basking frequency count. We measured the river stretch that was surveyed using a laser range-finder (Nikon Laser 800) and estimated the number of turtles that should be in that stretch using a previous population-size estimate (2007 Leaf River population estimate; Selman and Qualls 2009b). For each hourly basking frequency count, we divided the total number of *G. flavimaculata* observed basking by the estimated number of turtles in the observed stretch of river. We used a one factor ANOVA to determine if the estimated percentage basking was equal across observed months (April-October). A one-factor ANOVA was also used to determine if the estimated percentage basking was equal throughout the day. Lastly, we plotted raw basking counts of males, females, and juveniles observed to determine yearly basking frequencies, as well as seasonal basking frequencies for the Spring (April, May), Summer (June, July, August), and Fall (September, October).

Statistical Analysis of Population basking and environmental conditions.—We used a stepwise multiple regression to determine what effect environmental variables had on the number of turtles basking. In our first model, we analyzed percentage basking based upon water, air, and log temperatures using a 0.75 probability to enter and a 0.95 probability to leave the model. In our second model, we analyzed percentage basking based on air/water, log/air, and log/water temperature differences using a 0.75 probability to enter and a 0.95 probability to leave the model.

We also used a second order polynomial regression to determine the relationship between the number of turtles basking among males and females (using raw data from basking frequency counts) with water, air, and log temperatures. Significance level was determined if $p<0.05$ and we used JMP 7.0.2 (SAS Institute Inc., Cary, NC) for all statistical analyses.

RESULTS

During our 7 month study (June-October 2007; April, May 2008), 186.1 total hours of basking observation were logged at the Leaf River site. During these observations, 1124 independent basking occurrences for *Graptemys flavimaculata* individuals (478 F/ 595 M/ 51 JUV) were documented and an additional 157 hourly basking frequency counts were made at the Leaf River site.

Individual Basking Behavior.—Yearly mean basking duration for *G. flavimaculata* (all sexes) at the Leaf River site was 38.4 minutes (n = 1124, SD = ± 49.9min.). Mean male basking duration was 36.2 minutes (n = 595, SD = ± 49.9 min., Range = <1 – 464 min.), mean female basking duration was 42.8 minutes (n = 478, SD = ± 51.0 min., Range = <1 – 336 min.), and mean juvenile basking duration was 23.5 minutes (n = 51, SD = ± 31.1 min., Range = <1 – 149 min.). Basking duration differed significantly among the sexes ($F_{2, 1118} = 7.22$, $p = 0.0008$), with female basking duration being significantly longer than both males and juveniles. Male basking duration was not significantly longer than juveniles. We also documented 120 individuals (10.7%) that basked for periods longer than 100 minutes, 17 individuals (1.5%) that basked over 200 minutes, and five individuals (0.4%) that basked over 300 minutes. The maximum basking duration observed was 464 minutes (1004-1748 hrs) by a male *G. flavimaculata* on 15 April 2008 when log temperatures (27.3°C - 33.2°C) were considerably warmer than air (13.0°C - 17.6°C) or water (16.4°C - 20.0°C) temperatures.

There was also a significant difference in turtle basking duration by month ($F_{6, 1117} = 3.4$, $p = 0.003$) with individuals basking for longer periods in October than in June and August, but not longer than April, May, July, or September (Figure 4.1). There were also significant differences between the sexes ($F_{2, 1117} = 5.75$, $p = 0.003$) with females basking longer than juveniles but not males and no difference between males and juveniles. The interaction term was not significant ($F_{12, 1117} = 1.09$, $p = 0.36$).

We found a significant difference in turtle basking duration by time of emergence ($F_{10, 1096} = 3.51$, $p = 0.0001$), with longer durations when turtles emerged to bask in the morning (0700 - 1100 hrs) and afternoon hours (1600 - 1800 hrs) relative to midday

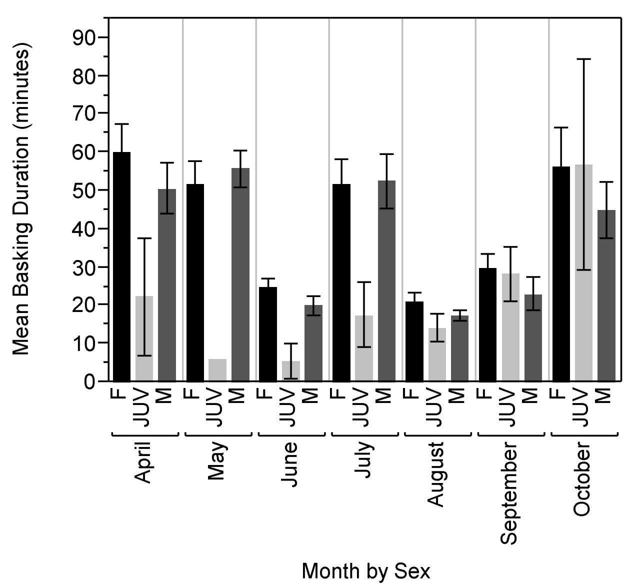


Figure 4.1. *Graptemys flavimaculata* basking duration (in minutes) by sex (Females- black bars, Males- dark grey bars, Juveniles- light grey bars) and month.

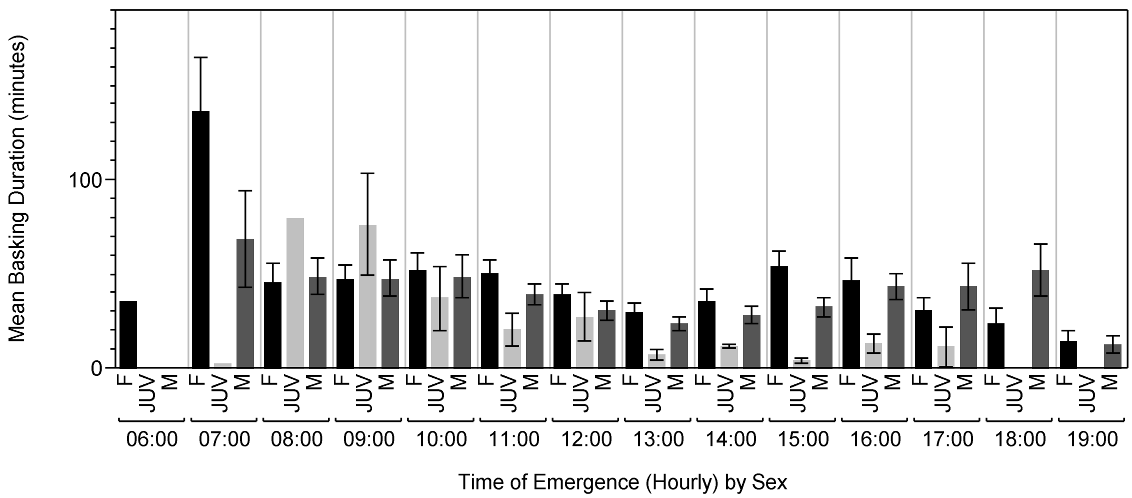


Figure 4.2. *Graptemys flavimaculata* basking duration (in minutes) by sex (Females- black bars, Males- dark grey bars, Juveniles- light grey bars) and daily time period. Each individual that emerged to bask within a specific hour (ex. 8:00-8:59 am) was considered only to emerge for that hour (08:00).

hours (1200 hrs- 1500 hrs; Figure 4.2). There was a similar significant difference among the sexes ($F_{2, 1096} = 5.22$, $p = 0.006$) with females basking longer than males and juveniles, and males basking longer than juveniles. There was not a significant interaction term ($F_{20, 1096} = 1.07$, $p = 0.37$).

We found a significant difference among the sexes in basking structure type used by individuals ($\chi^2 = 174.12$; $p < 0.0001$). Females almost exclusively used logs/floating logs as basking platforms (81.5%), with branches used less often (14.7%). Males used logs/ floating logs (46.5%) and branches (39.8%) almost equally; males also used crowns (9.3%) and tangles (4.1%) more often than females (crowns- 3.3%, tangles- 0.4%). Juveniles were most often observed using branches (64.7%) and logs (27.5%) with a smaller proportion using crowns (7.9%). In most circumstances, the preferred basking locations of juveniles were within a few meters of the bank and often located in or among overhanging vegetation/limbs. On one occasion an individual was noted to bask on the river bank (a female), but this was during high river levels when many deadwood structures were submerged.

Population Level Basking Behavior.—We found a significant difference in the percentage of all turtles basking across months ($F_{6,\,156} = 7.10$, $p < 0.001$; Figure 4.3) with significantly higher percentages basking during the month of May and July in comparison to the other months. We also found a significant difference in the percentage basking by time of day ($F_{13,\,155} = 1.89$, $p = 0.036$; Figure 4.4) with a higher percentage of the population basking from 1200-1400 hrs and again from 1600-1800 hrs relative to morning (0800-1100 hrs) and evening (1900-2000) basking percentages. Distinct seasonal differences were also observed (Figure 4.4, B-D) with significantly different basking percentages during the spring ($F_{10,55} = 2.46$, $p = 0.02$), when basking percentages were greater at midday relative to early morning and late evening hours. No differences were observed in basking percentage by time of day during the summer ($F_{13,70} = 0.85$, $p = 0.60$) or fall ($F_{11,31} = 1.49$, $p = 0.22$).

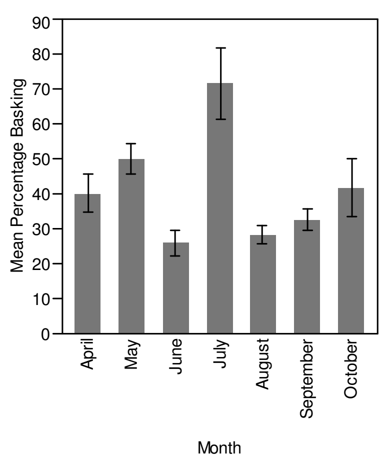


Figure 4.3. Percentage of the Leaf River *Graptemys flavimaculata* population basking across observed months.

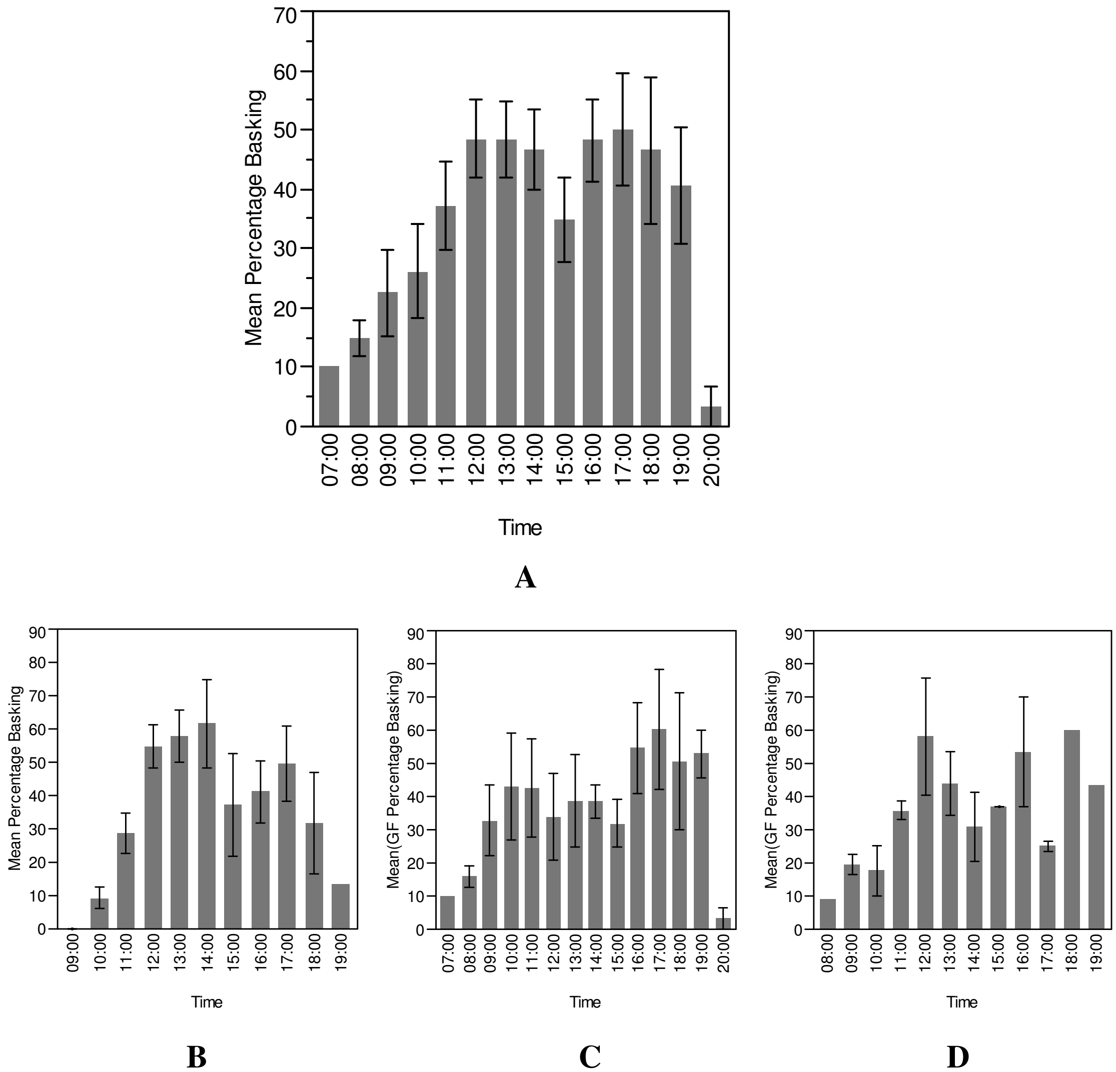
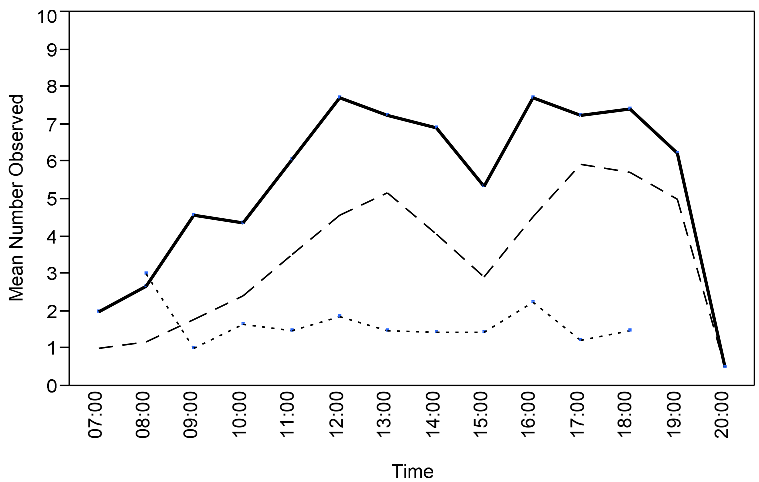
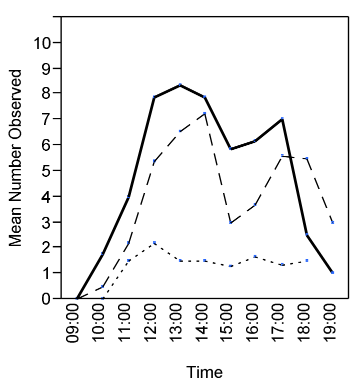


Figure 4.4. Percentage of the Leaf River *Graptemys flavimaculata* population basking during the daily activity period for the A) year, B) Spring, C) Summer, and D) Fall.

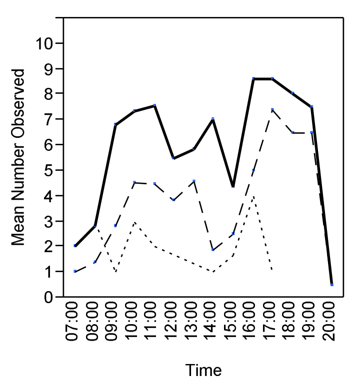
For both males and females, raw basking counts showed a clear yearly basking frequency pattern with a bimodal basking trend with peaks around 1200 - 1300 hrs and 1600 - 1800 hrs (Figure 4.5A). However, there were clear differences among the three seasons, similar to our basking percentage results. During the spring (Figure 4.5B; April, May), basking frequency was unimodal and peaked for both sexes during the midday hours (1200-1400 hrs); female to male basking ratio was higher during the spring than any other season. During the summer (Figure 4.5C; June, July, August), basking frequency was bimodal for both sexes with a morning peak around 0900 hrs and a late afternoon peak at 1600 - 1900 hrs. During the summer, basking occasionally continued until 2000 hrs (past sunset) and male basking counts were higher than female counts during all hours. During the fall (Figure 4.5D; September, October), basking frequencies



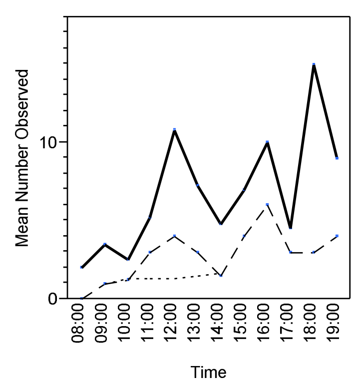
A



B



C



D

Figure 4.5. Leaf River *Graptemys flavimaculata* basking frequencies (raw data) of males (solid line), females (dashed), and juveniles (dotted) throughout daily hours during the entire year (A; April-October), spring (B; April and May), summer (C; June, July, August), and fall (D; September and October).

were less distinct with a slightly bimodal frequency for males and females with peaks at both 1200 and 1600 hrs. Female to male basking ratios were lower during the fall in comparison to other seasons. Juveniles basked at much lower levels throughout the year, with no clear pattern to preferred basking times.

Population Basking and Environmental Conditions.— Since basking was observed during all months under extremely variable environmental conditions, we wanted to determine which environmental variable(s) was responsible for most of the variation in population level basking. We found that there was little explained variation within our first stepwise multiple regression model, with air ($F = 0.115$, $R^2 = 0.0174$) and water ($F = 0.936$, $R^2 = 0.0166$) temperatures explaining most of the variation in percentage basking. The second model explained even less variation with the log/water temperature difference ($F = 0.784$, $\mathrm{R}^2 = 0.0051$) responsible for most of the variation. There was also a significant but low level polynomial relationship between basking male counts and log temperatures (males $R^2 = 0.053$, $p = 0.024$), but no relationship with air (males $R^2 = 0.011$, $p = 0.45$) or water (males $R^2 = 0.009$, $p = 0.51$) temperatures. Female basking counts showed significant but low level relationships with all three variables, including log ($R^2 = 0.13$, $p = <0.0001$), water ($R^2 = 0.065$, $p = 0.009$), and air ($R^2 = 0.064$, $p = 0.010$) temperatures.

Graptemys flavimaculata were also seen basking during many ‘unconventional’ conditions, including heavy cloud cover, rain, impending thunderstorms, and even into the late evening after sunset. The latter is of particular interest since turtles were observed during the summer to bask until after 20:00 hrs. During these periods, the ambient air and log temperatures were cooler than water temperatures and during the summer months, many turtles were also noted to be shedding carapacial scutes.

Disturbances While Basking.—Both natural and unknown disturbances prematurely startled turtles from their basking structures during the study; these instances disturbed 73 of 1124 (6.6%) monitored *Graptemys flavimaculata*. Most of the natural disturbances observed were of other turtles climbing onto the basking structures. Primarily, larger turtles pushed smaller ones off of their basking structures, both intraspecifically (mostly larger *G. flavimaculata* females disturbing males) and interspecifically (Pascagoula Map turtles- *Graptemys gibbonsi*, River Cooters- *Pseudemys concinna*). Other natural disturbances to turtle basking behavior include several different species of large predatory birds flying over (Osprey- *Pandion haliaetus*, Great Egret- *Ardea alba*, Great Blue Heron- *Ardea herodis*, Snowy Egret- *Egretta thula*), a swimming beaver (*Castor canadensis*), an unknown fish species that breached the water surface near basking turtles, floating logs, and floating river foam. An alligator (*Alligator mississippiensis*) was also observed to disturb basking *G. flavimaculata* at another site in a related study. Several species did not disturb basking turtles including swimming wood ducks (*Aix sponsa*) and water snakes (*Nerodia* sp.). Occasionally, turtles would abandon their basking site in the absence of any noticeable natural or human disturbance. This often occurred when there were large basking aggregations of turtles, with the whole bale of turtles diving into the water, most likely induced by one individual reentering naturally.

In addition to natural disturbances, 119 (10.6%) of the monitored *G. flavimaculata* were disturbed by human recreation/recreational boating. However, many of these instances were because of the researcher (74 observed turtles, 6.5%) when thunderstorms were approaching the site and precluded watching observed turtles until natural submergence. If these instances are removed, only 45 (4%) observed turtles were disturbed at the Leaf River site due to human activities/recreation. Only nine recreational boats were documented passing through the study site during our observation periods, but during these instances, almost all monitored basking turtles were disturbed (91%; 30 of 33 monitored individuals). Other human disturbances were mostly associated with neighboring landowners visiting the river to swim or walk the sandbars. A dog and horse on the cutbank also disturbed basking turtles, while some man-made floating objects also disturbed basking *G. flavimaculata* at a related study site (Selman et al. 2009).

Other unique basking observations.—On three occasions, extensive shuttling behavior was observed by the same individuals (one female, one juvenile female, and one male) which could only be determined by the unique coloration and/or patterning of the carapace. On 20 June 2007, a female was noted eight times shuttling on and off the same log between 1343-1511 hrs for a total basking time of 74 minutes (17 minutes, 13, 10, 8, 5, 7, 8, 6); unfortunately, no environmental data was collected on this day but we presume the water temperature was much cooler than log/air temperatures. On 10 September 2007, a juvenile female was observed shuttling eight times on and off different branches and tangles between 0857 (water: 26.9°C, air: 26.6°C, log: 28°C) - 1400 (water: 29.4°C, air: 32.2°C, log: 33.6°C) for a total basking time of 163 minutes (80, 9, 52, 19, 8, 8, 29, 31). On two occasions the juvenile female was disturbed by another turtle pushing her off the basking structure. Lastly, a male individual was noted on 8 April 2008 to shuttle 9 times between a log and floating log between 0939 (water:

20.1°C, air: 19°C, log: 22.5°C)- 1305 (water: 24.4°C, air: 26.2°C, log: 32.4°C) for a total time of 147 minutes (3, 2, 17, 9, 39, 2, 1, 2, 72).

Basking turtles were also observed on three occasions "testing" the water temperature before submerging after basking. This behavior was observed twice on 17 July 2007 with two different individuals (one male, one female). These two individuals, before they returned to the water, both dipped only their heads completely under the water surface. The male did this twice at 1020 hrs (water: 24.7°C, air: 28.3°C, log: 31.4°C) and thereafter returned to the water after basking for 141 minutes; the female did this once for approximately 15-20 sec (1808 hrs; water: 26.5°C, air: 27.1°C, log: 27.7°C) before submerging after basking for 138 minutes. During the week prior to these observations, several inches of rain fell within the Leaf River basin causing water levels to be much higher than normal and water temperatures to be unseasonably cool for July. Another observation of this behavior was made on 7 May 2008 (1727 hrs; water: 24.7°C, air: 27°C, log: 30°C) when an unmonitored male *G. flavimaculata* (did not observe emergence time for individual) submerged the anterior portion of his body while the posterior end was clinging to the basking structure. During the 1-1.5 minutes of this behavior, he raised his head out of the water twice and only thereafter did he submerge.

DISCUSSION

The difference in basking duration between adult and juvenile *Graptemys flavimaculata* is not surprising due to the dramatic differences in body sizes as well as the greater ability to lose/gain heat by smaller turtles (Boyer 1965). Our findings are similar to those of Auth (1975) who found that larger *Trachemys scripta scripta* basked for longer periods than smaller turtles. However, in our study, it is puzzling that males did not bask for much shorter periods in comparison to females (mean 6.6 minutes shorter throughout the year) considering the dramatic sexual size dimorphism in this species, with adult males as small as one-tenth the mass of adult females (Selman and Jones, 2010). One explanation is that while basking, males appeared to be better able than females to change their positions on basking structures because of their smaller size and greater agility. This presumably allows them to regulate their temperatures on a finer scale than females by changing their body position relative to the sun, thus permitting them to bask slightly longer than expected; Bulté and Blouin-Demers (2009) also suggested that female *G. geographica* do not thermoregulate as accurately (i.e., their body temperatures were less often within preferred body temperatures) relative to males and juvenile females. During this study, maximum basking durations found for individuals (464 minutes for a male, 336 for a female) were much longer than previously reported in other aquatic Emydid turtles (Auth 1975), but not longer than our other recent studies that documented similar maximum basking durations for two other aquatic turtle species (*Pseudemys concinna*- 485 minutes, Selman and Qualls 2009c; *T. s. scripta*- 430 minutes, Selman and Qualls 2009d).

Daily and seasonal differences in basking durations should be expected due to seasonally changing environmental conditions and physiological needs of turtles. During the spring months, water temperatures are cooler than air and log temperatures, making basking critical for increasing turtle body temperatures. During the spring, we observed the longest durations and highest basking frequencies of males and females, as well as the highest population level basking percentages, which were also observed in *Graptemys* *nigrinoda* (Waters 1974). During this time, female basking durations are also longer, likely due to timing of ovarian follicle maturation prior to the nesting season (Shelby et al. 2000, Horne et al. 2003) and larger females take longer to heat than smaller males (Weathers and White 1971). Therefore, during the spring basking is likely more utilized for meeting thermoregulatory needs.

By summer, water temperatures have increased substantially (up to 35°C) and basking for thermoregulation appears to be negligible. Intense solar radiation during summer midday hours limits basking durations since turtles could overheat which precludes shell drying during these hours. Since turtles bask for longer durations during the morning and evening hours of the summer and early fall, we hypothesize that basking during these periods is more for shell drying, conditioning, and scute ecdysis rather than primarily for thermoregulation. Manning and Grigg (1997) proposed that the basking behavior exhibited by an Australian side-necked turtle (*Emydura signata*) was not for thermoregulation due to thermoconformy of turtle body temperatures to environmental temperatures; they suspected that basking in this species could be for vitamin D synthesis and/or removal of algal or fungal infestations.

One anomaly during our summer observations was the month of July 2007, when basking durations (Fig. 4.1) and basking percentages (Fig. 4.3) were much longer and higher, respectively, than other summer months. However, July air and water temperatures were unseasonably cool, similar to those of April and May, while river levels were unusually high. Additionally, the week prior to observations was dominated by large amounts of rain and was not conducive to turtle basking. Therefore, the long basking durations observed during this month were likely abnormal for this time of year, but quite similar to Waters (1974) observations, who found basking activity of *G. nigrinoda* to be more intense following several days of unfavorable basking conditions.

Fall basking appears to be similar to spring basking in that water temperatures begin to cool while air and log temperatures during the midday continue to be conducive to turtle basking. Also, male turtles are likely undergoing spermiogenesis during the fall (Shelby and Mendonça 2001) leading to longer basking durations and higher basking frequencies. Even though females have longer basking durations during the fall, they bask in lower frequencies in comparison to spring months, thus making it unclear if females begin follicular growth for the upcoming nesting season during the fall (Shelby et al. 2000). While no data was collected over the winter months, Jones (1996) and Moore (2003) observed *G. flavimaculata* active during all months of the year, including winter months; we also opportunistically noted turtles basking during the winter while retrieving temperature data from data loggers with much lower numbers observed basking compared to the spring to fall. We presume that when turtles are active during the winter, they may bask for long durations due to decreased solar radiation and lower water temperatures that would promote long basking periods.

Little is known about the life history of juveniles for most turtle species, with only two studies documenting basking behavior in juvenile *Graptemys* (Waters 1974, Lindeman 1993). While basking, juveniles face a difficult challenge: basking increases the ability to assimilate food for growth which is key for this life history stage, but it also increases the potential for predation. One would presume that the smaller size and surface area to volume ratio of juveniles with their greater ability to lose/gain heat, would lead to shorter basking durations than adults. For the 51 documented *G. flavimaculata* juvenile basking durations, we found shorter basking times for juveniles relative to adults, but some basking durations were much longer that expected for this age class (max: 149 minutes). In addition, basking durations for juveniles are longest during the morning hours and decrease throughout the day (similar to our daily basking frequencies results), making juvenile basking more unpredictable than adults. Waters (1974) also noted juvenile *G. nigrinoda* basking to be erratic, with most occurring primarily during the morning hours. Juvenile *G. flavimaculata* often chose basking locations closer to the bank and basking structures were usually located in or among overhanging vegetation/limbs. We presume that juveniles choose locations that are closer to the bank and in shallower water to avoid larger aquatic predators (primarily predatory fish), while also choosing to bask among branches/overhanging vegetation to avoid predatory birds. These areas may also provide dappled sun and shade, allowing juveniles to shuttle between sunny and shady conditions. The poorer swimming ability of juveniles may also confine them to these areas with slower current speeds near the bank relative to the main river channel.

Different sexes did not use basking structures equally, likely due to their dramatic size differences, with females requiring more supportive structures (logs) relative to males or juveniles. However, our study cannot account for two factors: 1) the changing matrix and availability of basking structures in this dynamic riverine system and 2) the percentage of each structure type occupied by basking turtles. Even though we found a large number of females using logs and males using branches, we cannot account for the availability and/or the percentage of each type of basking structure used within our river system throughout the entire year since these variables were not measured. Lindeman

(1999) addressed this topic with deadwood surveys among multiple river systems of the southeastern United States and found that within the Pascagoula River system the most common type of substrate was branches, followed by logs, tangles, stumps, and tree crowns. Branches were rarely occupied by basking turtles (5%) while logs (12%) and the least encountered substrate type, tree crowns (15%) were occupied most; tree crowns were also highly occupied by turtles within the Pearl (45%) and Tennessee (96%) river drainages. Therefore, if present, turtles may choose tree crowns that offer different basking structure sizes (limbs vs. trunk), different angled structures for differences in daily solar radiation needs, better protection from aerial predators, and safety in numbers since many turtles can bask in close proximity to each other.

Lindeman (1999) also stated the importance of deadwood to basking turtles and that “de-snagging” operations would have a detrimental effect on turtles. In addition to de-snagging, poor forestry practices within riparian corridors and near stream banks could have a deleterious impact on riverine deadwood abundance. Forestry operations have been observed on multiple occasions to cut all trees from the river banks (W. Selman, pers. obs.), thus leaving no future input of basking structures (i.e. trees falling in as river erodes outer cutbank) and no support for the bank soil (Selman and Qualls 2009b). These practices lead to increased sedimentation, accelerated filling of deep water channels preferred by this species, and total dependence of that reach on upstream sources for basking structures.

The absence of a strong correlation between *Graptemys flavimaculata* basking and the yearly environmental (temperature) variables we recorded indicates the dramatic difference in seasonal, daily, and individual turtle needs. Ecksdine (1985) conducted surveys on *G. geographica* in Michigan and found the most important predictor of turtle basking was the temperature difference in basking site and water which explained 26.5% of the daily basking variation, with basking site temperature explaining another 16%. However, in comparison, our study encompassed almost the entire active season (April-October) and the yearly variation in environmental variables is likely much different than the three month period observed by Ecksdine (1985). In contrast, Coleman and Gutberlet (2008) found low level polynomial relationships, similar to our study, in *G. ouachitensis sabinensis* and *G. pseudogeographica kohnii* basking in comparison to environmental temperatures from the Sabine River in southeast Texas. Turtle basking was also greatest when water temperatures were between 15°C -25°C for both species examined, similar to our findings.

One aspect of basking that cannot be addressed by our study or previous studies is the use of basking as a means for turtles to rest in flowing environments. Very few studies mention the need for turtles to rest by using basking as the means to accomplish this (Boyer 1965, Waters 1974). In non-flowing conditions, turtles may float at the water surface without expending much energy and rest without having to bask aerially. In contrast, turtles in flowing conditions are constantly swimming upstream in order to maintain connection to home ranges, while also contending with water currents while feeding (Waters 1974). We suspect that some of the variation that cannot be explained by our analyses is due to individuals basking to rest. This would need to be verified in future studies.

The impact of natural and anthropogenic disturbances appears to be minimal in this population. Larger aquatic, aerial, and terrestrial predator or "predator-like" species appeared to have the biggest impact on whether turtles were disturbed, but these were very limited encounters. Because this is a relatively isolated population and within a small riverine system, when human disturbance occurs, it disturbs a high percentage of the basking turtles relative to other sites where turtles may be more acclimated to human presence. In contrast, other *Graptemys* populations in high recreational use areas appear to be somewhat habituated to human activities and very few are disturbed while basking (W. Selman, pers. obs.; P. Lindeman and B. Anders, pers. comm.). Further research is needed to determine what role disturbance plays in turtle behavioral and physiological responses.

Throughout this study, we observed many unique behaviors of *G. flavimaculata*, as well as other turtle species, by studying basking behavior in a more classical, field-based approach. However, we believe that many questions are still to be answered related to this topic and future studies should utilize more advanced technological tools (i.e. temperature sensitive transmitters/loggers) to answer the complexities of this behavior. In addition, calculating basking percentages based on known population estimates of an observable, measured river stretch may allow future researchers to answer complex questions, especially when trying to compare populations of sympatrically occurring basking turtle species; this may, however, have some limitations if confidence limits are markedly different between the two populations. In summation, the basking ecology of this species and turtles in general, appears more difficult to explain than previously thought due to complex daily individual physiological demands across seasons, sexes, and reproductive conditions, as well as environmental conditions that dramatically fluctuate across seasons. Our conclusions sound very similar to what Moll and Legler (1971) eloquently stated almost forty years ago: that basking is likely initiated by a single impulse, which is either triggered by an external factor or internal physiological need, while other secondary benefits are thereafter gained.

CHAPTER V

THE IMPACTS OF HUMAN RECREATION ON THE BEHAVIOR AND PHYSIOLOGY OF THE IMPERILED YELLOW-BLOTCHED SAWBACK (*GRAPTEMYS FLAVIMACULATA*)

Abstract.—The impact of human disturbance on endangered animal populations is becoming an area of increasing interest due to the growing use of wildlands by humans for recreation. Very few studies have documented the impact of human disturbance on animal behavior and physiology, with no current studies existing for any turtle species. Turtles are one of the most endangered taxonomic groups and many are of conservation concern, including the Yellow-blotched sawback (*Graptemys flavimaculata*), a freshwater turtle of the Pascagoula River system, Mississippi, USA. We studied *G. flavimaculata* individual and population level basking behavior, while also documenting the impacts of human recreation on basking behavior at a recreationally disturbed site and a control site. We also assessed the physiological response of turtles to human disturbance by measuring shell condition, as well as measuring baseline corticosterone and corticosterone stress response of captured turtles at the two sites. Our results indicated that turtles basked for significantly shorter durations and populations basked at significantly lower percentages at our recreationally disturbed study site. Larger and slower boats disturbed a significantly higher percentage of basking turtles in comparison to smaller and faster watercraft. However, we found no difference in baseline corticosterone levels or corticosterone stress response between the disturbed and control site, but did find that males had a significantly higher corticosterone stress responses relative to females. Conversely, shell condition was significantly worse at the

recreationally disturbed site with many individuals exhibiting scute fungal infections. The impact of recreational boating on turtles from our disturbed site has likely grown over the last 22 years due to an increase in the number and size of boats that use the river. This trend will likely continue unless some restrictions are enacted to limit the size of the boats that access the river.

INTRODUCTION

Human disturbance of wildlands is increasing (Boyle and Samson 1985, Flather and Cordell 1995) and is implicated as one of the top five leading causes for the decline of threatened/endangered species within the United States (Czech et al. 2000). Even though it is a leading cause of species endangerment, the impact of human disturbance on turtle populations has been largely neglected except for a handful of recent studies (Burger and Garber 1995, Garber and Burger 1995, Krzysik 1997, Moore and Seigel 2006, Bowen and Janzen 2008, Bulté et al. 2009). Most of these studies found negative impacts of human disturbance on turtle populations, turtle habitat, and turtle behaviors, but virtually nothing is known about the physiological response of turtles to these likely novel ‘stressors’ in their environment.

The conservation of tortoises and freshwater turtles has garnered much attention in the last decade (Buhlmann and Gibbons 1997, Klemens 2000, Moll and Moll 2004), particularly due to the increased rate of turtle habitat destruction/alteration on a global scale (reviewed in Mitchell and Klemens 2000), increased human consumption of turtles in the Asian traditional medicine/food markets (Moll 1982, Chen et al. 2000, Thorbjarnarson et al. 2000, Van Dijk 2000, Van Dijk et. al. 2000, Moll and Moll 2004), and the increased number of turtles offered in the pet trade (Thorbjarnarson et al. 2000, Van Dijk et al. 2000). These three mounting factors have led chelonians to be one of the most endangered taxonomic groups of the animal kingdom, with 132 of the 212 (62%) species assessed by the World Conservation Union (IUCN) listed as critically endangered, endangered, or vulnerable (http://www.iucnredlist.org/static/stats, Table 4a; accessed 18 November 2008). In the near future, it is likely that many more turtle species will be listed or upgraded as new population data becomes available.

Since aerial basking is one of the most commonly observed behaviors of many aquatic turtles, it is easy to assess the impacts of human recreational disturbance on this behavior. Aerial basking is critical for many species of turtles to maintain many physiological activities (Boyer 1965), including metabolism (Moll and Legler 1971), vitamin K synthesis (Pritchard and Greenhood 1968), shell conditioning/scute ecdysis (Selman and Qualls, unpubl. data), removal of ectoparasites (Cagle 1950, Neill and Allen 1954, Vogt 1979, Selman et al. 2008, Selman and Qualls 2009a), and reproductive function (Hammond et al. 1988). However, when turtles are prevented from basking and achieving desired thermoregulation, this could lead to many physiological consequences: altered levels of circulating steroid hormones due to elevated levels of the stress hormone, corticosterone (Licht 1984, Licht et al. 1985, Moberg 1985, Greenburg and Wingfield 1987, Mahmoud et al. 1989, Sapolsky et al. 2000); lowered internal body temperature preventing optimal metabolic activity (Boyer, 1965); possible immune suppression due to chronically elevated corticosterone levels (Apanius 1998, Sapolsky et al. 2000); and potential impairment of the reproductive system and ability for females to yolk developing ova (Mahmoud et al. 1989, Viveiros and Liptrap 1995).

The Yellow-blotched sawback (*Graptemys flavimaculata*) is a riverine turtle that is endemic to the Pascagoula River system of southern Mississippi, USA. Populations also occur outside of the Pascagoula River, including its chief tributaries, the Leaf, Chickasawhay, and Escatawpa Rivers, as well as other smaller creek systems (Cliburn 1971, U.S. Fish and Wildlife Service 1993, Ernst and Lovich 2009, Selman and Qualls 2009b). *G. flavimaculata* was originally state listed as endangered in the 1970's (R.L. Jones, pers. comm.) and observed population declines led to federal listing as a threatened species in 1991 (U.S. Fish and Wildlife Service 1991). Following these declines, Horne et al. (2003) found that female *G. flavimaculata* from the Pascagoula River had a lower reproductive frequency in comparison to other similar species of *Graptemys*. Others also found irregular sex hormone levels of this population in comparison to an upstream reference population (Shelby et al. 2000, Shelby and Mendonça 2001).

One aspect that was addressed, but not implicated in the impacts/declines, was the substantial increase of recreational boating in the Pascagoula River. This area is the widest portion of the Pascagoula River system and multiple boat ramps make it readily accessible for human recreational purposes. Moore and Seigel (2006) found that during 118 hours of basking observation, 210 human disturbances were noted in the Pascagoula River. Also, approximately 50% of boat passing events caused *G. flavimaculata* individuals to abandon their basking site and females were more prone to abandon their basking sites than males (Moore and Seigel 2006). Therefore, if many turtles are not basking for the desired duration and if females are more likely to abandon their basking sites than males, could this then alter natural thermoregulatory behaviors and likely have dramatic physiological consequences? Could these consequences include the low reproductive output and irregular sex hormone levels previously found in the Pascagoula River population (Shelby et al. 2000, Shelby and Mendonça 2001, Horne et al. 2003)? In this study, we sought to determine: 1) the average individual basking duration and population-level basking frequency of turtles in a disturbed population compared to a relatively undisturbed population, 2) the disturbance rates and disturbance types in the two monitored populations, 3) the impact of human disturbance on the physiological condition of turtles (i.e., shell condition and stress response) within the two populations, and 4) the historical and present boating trends in the Pascagoula River.

MATERIALS AND METHODS

Basking Observation Methods.—Basking behavior of *G. flavimaculata* was studied at a recreationally disturbed study site (Pascagoula River site near Vancleave, Jackson County, MS) and an undisturbed site (Leaf River site near Hattiesburg, Forrest County, MS; see chapter IV). Observations of turtle basking behavior were conducted at both sites during the months of June 2007-October 2007 and April-May 2008. At least two days of observation (one weekend and one weekday) were attempted at each site during each month and observations were made throughout turtle basking hours (i.e. 0700 hrs-1900 hrs during the summer); weather conditions sometimes did not allow for complete observations at each site during every month. Daily observations almost always lasted 4 hrs, but did not exceed 10 hrs in a single observational day. The observer was located in a concealed position, either on a sandbar or inside of river bend, opposite of a cutbank section where turtle basking structures were abundant. Observations were conducted with binoculars and/or a 60mm, 15 - 45 power spotting scope. Basking behavior was studied predominantly on mostly sunny to sunny days by two methods: individual turtle basking observations and hourly basking frequency counts. The first method was used to document individual basking duration, whereas the second method was used to determine daily species basking frequency.

In the first method, when an individual *G. flavimaculata* was spotted emerging onto a basking structure, the sex of the turtle (if possible), time of emergence, and snag type were recorded; when the individual terminated basking, the time of submergence and total basking time was recorded. If submergence was due to a natural or human disturbance, this was also noted as either 'yes' or 'no' (see below). We could not confirm the identity of every individual when basking (unless a turtle had unique markings) and therefore could not determine the amount of time during the day that an individual basked. The second method, hourly basking frequency counts, was also conducted during these same observation periods by counting the number basking *G. flavimaculata* within a measured, predetermined stretch of viewable river at the beginning of every hour. Sex of individuals was recorded when possible, as well as the number of other turtle basking species observed.

Due to the Poisson distribution of individual basking duration (i.e. with many more individuals basking for a short period of time and fewer basking for longer periods of time), we log-transformed our data to meet parametric assumptions. We then used a *t*-test to determine if turtle basking durations were equal between the two study sites using males and females together, as well as analyzing males and females separately to determine if there were sex-specific differences in basking duration. In addition, we used a one factor analysis of variance (ANOVA) to determine if mean basking durations of turtles were equal for Leaf River turtles, undisturbed Pascagoula River turtles, and disturbed Pascagoula River turtles; this analysis was done to determine if undisturbed Pascagoula River turtles wanted to achieve similar basking durations as Leaf River turtles. We also used a two factor ANOVA to determine if mean basking time of turtles (dependent variable) was equal for the two study sites (factor) by the time of emergence (factor). For the time of emergence, we included all turtles that emerged within a specific hour (ex. 11:00 am to 11:59 am) to occur only at the beginning of that hour (ex. 11:00) to simplify our data analysis. A chi-squared contingency table analysis was used to determine if males and females were disturbed at equal frequencies.

Due to differences in population sizes between sites and the inability to standardize for this in our hourly basking frequency counts, we decided to calculate the percentage of the population that was basking. We completed this by measuring the length of river stretch that was surveyed at each site and estimating the number of turtles that should be in that stretch using a known population estimate from 2007 (Selman and Qualls 2009b). Thereafter, for each hourly basking frequency count, we divided the number that we observed basking by the estimated number of turtles that should be in the observed stretch of river. Our basking percentage data also exhibited a Poisson distribution and was therefore log transformed to meet parametric assumptions. For our data analysis, we used a two factor ANOVA to determine if population level basking percentage (dependent variable) was equal throughout the day (factor) and among the two sites (factor).

Quantifying Human Disturbance.—During basking observations of individual turtles, human and natural disturbances were recorded. Natural disturbances were recorded if basking was terminated prematurely due to a natural event (i.e. a large bird flying over basking turtles). Human disturbance was recorded at each site, but was prevalent at the Pascagoula River site where recreational disturbance by boats was high. When a boat passed our observation point, the following was recorded: time of boat passing, relative size of boat, time between human disturbances, and number/percentage of viewed turtles disturbed; the percentage of turtles disturbed was calculated by dividing the number of individual turtles that were disturbed by a passing boat/boat wake and by the total number of basking turtles that were being monitored at the time of disturbance. The size of passing boats was qualitatively determined and were categorized into the following groups: small boat (SmB)- primarily 'jon boats' with small outboard motors (usually <40 horsepower); medium boat (MdB)- mostly 'bass boats' and other mid sized boats that did not produce a large wake; large boat (LgB)- pontoon boats, ski boats, offshore boats, and all other large-sized boats that produced a sizeable wake; and personal water craft (PWC). It was also noted if these boats were going at a slow speed since it has been noted in previous studies that more turtles are disturbed by boats passing at a slower speed relative to faster boats (Moore and Seigel 2006).

Due to the abundance of shorter times between disturbances in comparison to longer times between disturbances, we log-transformed our data to meet parametric assumptions. To quantify the impact of disturbance, we used a one factor ANOVA to determine if mean time between disturbances was equal for the Leaf and Pascagoula River sites. We also used a one factor ANOVA to test to see if the time between disturbances was equal across all days at the lower Pascagoula River site. A non-parametric Wilcoxon Rank Sums test was used to determine if the percentage of observed turtles disturbed per passing boat was equal for different boat sizes and speeds at the lower Pascagoula River site.

Sample Collection and Corticosterone Assay.—G. flavimaculata were sampled for seven months (April-October) in 2007 and 2008. Trapping was conducted at the same two sites where our basking observations were made: Leaf River site (recreationally undisturbed) and Pascagoula River site (recreationally disturbed). Turtles were trapped by submerging basking traps (made of ¾" PVC coated crawfish wire) from turtle basking structures or by swimming and dip-netting basking/swimming turtles during low river levels at the Leaf River site. To ensure that we received a true baseline corticosterone value, we only included a turtle if we saw it jump into our basking trap/dip net and turtles were always bled within five minutes of capture. For a more detailed description of trapping methods see Selman and Qualls (2009b).

Immediately following capture, 1 mL of blood was collected from female *G. flavimaculata* and 0.6-0.75 mL from males from the coccygeal vein using a heparinized 1mL syringe and a 26 ½ gauge needle. Following this blood sample (hereafter referred to as baseline), individuals were placed into a 18.9 liter bucket (5 gallon) with approximately 5 cm of river water (to prevent overheating, especially during the summer months). The bucket was covered with a bucket lid and 35 minutes after the initial sample collection, we took another 0.1 mL (time 35) blood sample. Blood samples were stored on ice for 4 to 6 hours, and then centrifuged. Plasma and blood cells were separated, and thereafter, frozen. When possible, blood was collected from five adult males and five adult females per site during each month from April to October. Following capture, sex and typical morphometric data were collected.

Plasma corticosterone was measured using a direct radioimmunoassay (RIA) (Wingfield and Farner 1975, Ball and Wingfield 1987, Ketterson et al. 1991). Briefly, approximately 2000 counts per minute (CPM) of H^3-corticosterone (PerkinElmer Life and Analytical Sciences, Massachusetts) was added to samples (for later determination of extraction efficiency) and samples were extracted three times using 4.5ml of diethyl ether. Following extraction and drying of samples under nitrogen gas, samples were reconstituted in 1ml phosphate buffered saline (PBS) solution with gelatin. Reconstituted samples were run in duplicate in a competitive binding immunoassay using radiolabeled corticosterone and an antibody specific to corticosterone (Fitzgerald Industries International, Inc., Massachusetts). Levels of corticosterone were determined from a nine point standard curve. Intra- and inter-assay variations were determined from a series of known standards run in conjunction with the samples. Intra-assay variation was 11.16% and inter-assay variation was 29%. Due to the large number of assays and large interassay variation, a correction factor was applied to each sample. To calculate our correction factor, the "grand" mean of all assay standards was divided by the individual assay mean; individual sample values were then multiplied by our correction factor (Jawor et al. 2007).

Additionally, many samples fell below the detection limits for the assay (91 of 492 samples); this was particularly evident for baseline samples (85 of 246). For our statistical analyses, these individual values were considered as "zeros" to provide more power to the analysis and more closely approach the "real" mean (to avoid positively biasing our sample means); we do however acknowledge that in reality there is some extremely low level of circulating corticosterone within these individuals, but it was outside of our assay detection limits. Lastly, to determine the stress response of individuals, we subtracted baseline values from time 35 values; the difference between these values is also expressed in ng/mL.

Since many baseline values were considered zeros and the distribution of these values was non-normal even after attempting to log-transform the data, we used our untransformed, raw baseline corticosterone data and analyzed it using nonparametric statistical analyses. A Wilcoxon Rank Sums test (chi-squared approximation) was used to determine if time zero corticosterone levels (ng/mL) were the same between the two sites for males and females using separate analyses. Then a two-factor ANOVA was used to determine if the stress response was equal by site, by sex, and with a sex-site interaction. For all statistical analyses, our significance level was $p < 0.05$.

Quantifying Shell Condition of Turtles.—The shell condition of captured turtles was assessed by observing the presence/absence of shell fungus, which occurs when scutes fail to shed properly. Shell fungus was determined 'present' if white spots were visible under/within carapacial scutes. We used a chi-squared contingency table test to determine if the frequency of shell fungus was equal among the two study sites. The severity of fungal growths was not quantified and included into our data analysis, but it will be discussed.

Assessing Historical Boating Trends in Lower Pascagoula River.—During our study, many local residents from the Pascagoula River area mentioned that the number and size of boats in the Pascagoula River has increased in the last 10-15 years. Therefore, boat registration data was collected and compiled for a twenty-two year interval (1987-2008) for Jackson County, MS (by Gail Marshall, Mississippi Department of Wildlife, Fisheries, and Parks). We chose to use Jackson County since it is bisected by the Pascagoula River and Pascagoula River study site occurs in this county. This includes five two-year periods with five years between these intervals since boats are registered across multiple years (1987/1988, 1992/1993, 1997/1998, 2002/2003, 2007/2008). The boating information that is reported by individuals includes the length of boat to be registered and if the user plans to use it in either saltwater or freshwater conditions. We note that not all registered boats from Jackson County, MS utilize this portion of the Pascagoula River and also, that boats from other counties in Mississippi or from nearby Alabama may use this stretch of river. We then used these data to examine if there are any trends in this county for the number and size of boats registered, while also qualifying observations of local residents on boat traffic. These data could then be used to determine the impact recreational boating is currently having, and in the future, may have on turtle basking behavior and turtle populations.

RESULTS

Turtle Basking Behavior.—Throughout 7 months (June-October 2007; April, May 2008), 360.6 hours of basking observation were logged at both sites (186.1 hrs- Leaf River, 174.5 hrs- Pascagoula River). During these observations, 1124 independent basking occurrences for *G. flavimaculata* (478 F/ 595 M/ 51 JUV) were documented at the Leaf River site (undisturbed) and 1321 (483 F/ 787 M/ 51 JUV) at the Pascagoula River site (disturbed). Additionally, 297 hourly basking frequency counts were made at both sites (157-Leaf River, 140- Pascagoula River).

Mean basking duration for *Graptemys flavimaculata* at the Leaf River site was 38.4 minutes (SD ± 49.9min., including juveniles) with males basking for a mean of 36.2 minutes (SD ± 49.9 min., Range = <1 – 464 min.) and females basking for a mean of 42.8 minutes (SD ± 51.0 min., Range = <1 – 336 min.). *G. flavimaculata* at the Pascagoula River site had a mean basking duration of 26.5 minutes (SD ± 27.0 min.; including juveniles) with males basking for a mean of 26.9 minutes (SD ± 28.1, Range = <1 – 171 min.) and females basking for a mean of 25.8 minutes (SD ± 25.4, Range = 1 – 125 min.). *G. flavimaculata* basked significantly longer at the Leaf River site relative to the Pascagoula River site (t= 12.33, df = 1, p = 0.0005; Figure 5.1). Also, females from the Leaf River basked significantly longer than Pascagoula River females (t = 17.65, df = 1, p = <0.0001), whereas basking time for males did not differ significantly between the two sites (t = 0.94, df = 1, p = 0.33). Further, Leaf River turtles and lower Pascagoula River undisturbed turtles basked for longer durations than lower Pascagoula River disturbed turtles ($F_{2,2347}$ = 10.48, p <0.001), while there was no difference in mean basking duration

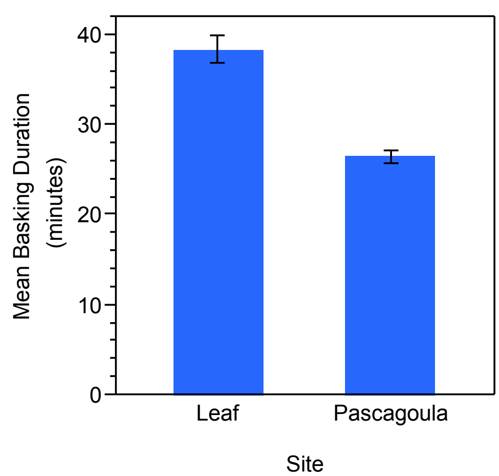


Figure 5.1. *Graptemys flavimaculata* basking durations between the two study sites. Numbers include male, female, and juvenile basking times. Error bars represent one standard error.

between Leaf River and undisturbed lower Pascagoula River turtles. In addition, *G. flavimaculata* females from the Pascagoula River site were more likely to be disturbed than males ($X^2 = 30.7$, df = 1, $p < 0.001$). When we compared the basking durations of turtles throughout the day among sites, we also found a significant difference in basking duration by site ($F_{1,\,2394} = 8.89$, $p = 0.0029$), as well as by time of emergence ($F_{11,\,2394} = 5.80$, $p < 0.0001$). The interaction was not significant ($F_{11,\,2394} = 0.94$, $p = 0.50$). *G. flavimaculata* basking durations were significantly longer at the Leaf River site relative to the Pascagoula River site throughout the day, and turtles at both sites basked for longer durations in the morning and afternoon hours in comparison to midday hours (Figure 5.2).

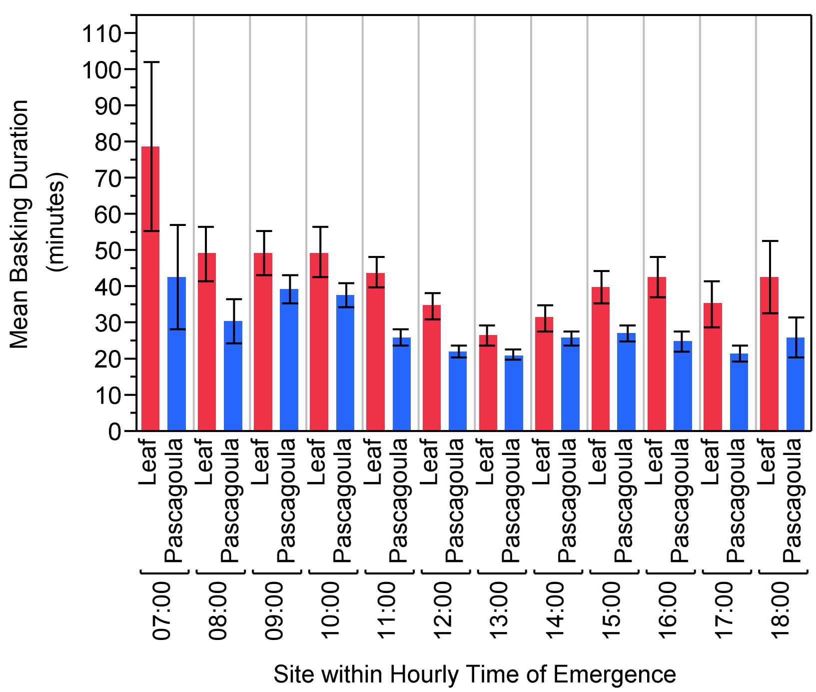


Figure 5.2. *Graptemys flavimaculata* basking durations by hourly time of emergence between the two study sites. Means include male, female, and juvenile basking times. Error bars represent one standard error.

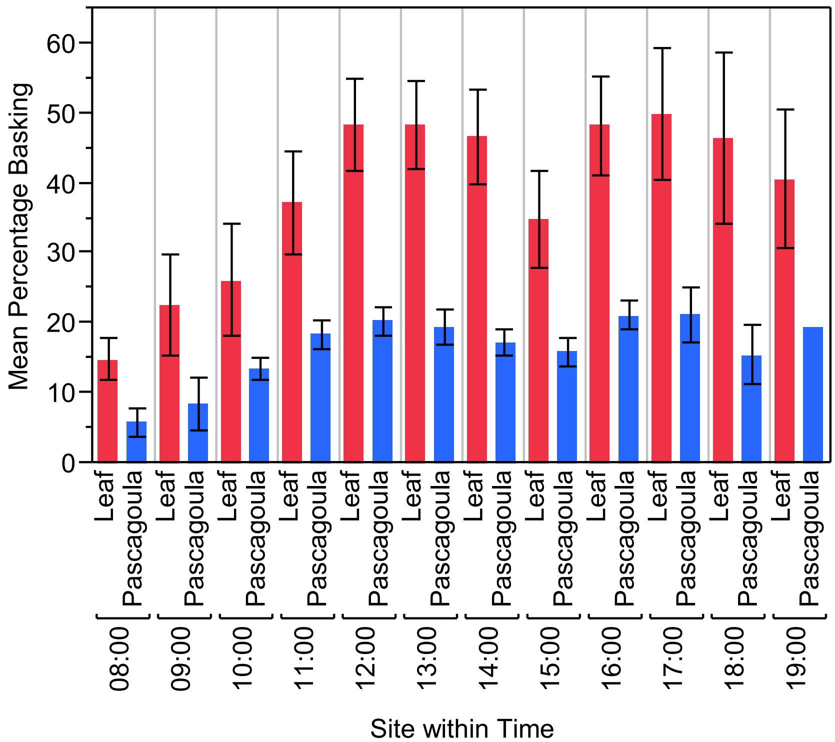


Figure 5.3. Mean basking percentages for *G. flavimaculata* throughout the day and between sites. Error bars represent one standard error.

Since there was little difference in our raw population basking numbers across months and between sites, we used basking percentage to compare between our two populations throughout the daily activity period. We found a significant site difference ($F_{1, 295} = 25.2$, $p < 0.001$; Figure 5.3) and a significant difference in basking percentages by the time of day ($F_{13, 295} = 2.62$, $p = 0.002$); there was no significant site by time interaction ($F_{13, 295} = 0.58$, $p = 0.86$). Turtle basking percentages were significantly higher at the Leaf River site relative to the Pascagoula River site, while basking percentages were higher during the midday and afternoon hours in comparison to morning basking percentages.

Impacts of Recreational Boating on Basking Behavior.—During 147.9 observational hours at the Leaf River site, we observed 45 human disturbances (19 by researchers) with a mean time between disturbance of 179.9 min. (SD ± 145.8 min., Range= 3-600 min.). Conversely, during 174.5 hours of observation at the Pascagoula site, 987 recreational boating disturbances (16 by researchers) occurred with a mean time between disturbance of 10.4 minutes (SD ± 21.8 min., Range = 0-312 min.). Mean time between disturbances was significantly higher at the Leaf River site relative to the Pascagoula River site ($F_{1,\ 887} = 198.22$, $p < 0.0001$; Figure 5.4). At the Pascagoula River site, there was also a significant difference in time between disturbance among days ($F_{6,\ 848} = 27.04$, $p = <0.001$) with midweek days (Tuesday, Wednesday, and Thursday; mean time between disturbances was >50 minutes) having significantly longer times between disturbance than weekend or weekend bordering days (Friday, Saturday, Sunday, and Monday; mean time between disturbance <20 min).

Of the 986 disturbances at the Pascagoula River site, we observed 363 small boats (26 were moving at a slow speed), 339 medium boats (20 slow), 191 large boats (32 slow), and 93 PWC's. Additionally, 529 of the 1321 (40%) monitored turtles were

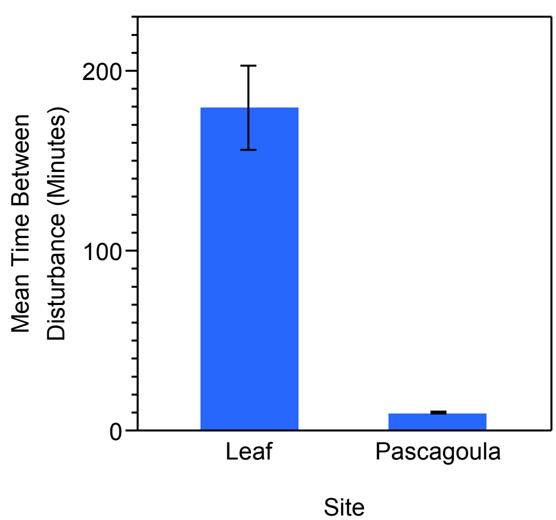


Figure 5.4. Mean time between human disturbance at the two study sites. Error bars represent one standard error.

disturbed by recreational boating and an additional 103 (7.8%) were disturbed by natural or unknown disturbances. A mean of 0.27 turtles were disturbed per small boat passing (9% of observed turtles disturbed per boat passing), 0.31 disturbed per medium boat (11%), 1.00 disturbed per large boat (32%), and 0.13 per PWC (6%). Additionally, 2.16 turtles were disturbed per slow small boat passing (50%), 1.8 per slow medium boat passing (56%), and 1.9 per slow large boat (62%). Percentage of turtles disturbed by different boat types and speeds was significantly different ($X_2 = 191.17$, $p < 0.0001$) with slow boats disturbing a higher percentage of turtles than fast boats and larger boats disturbing more turtles than smaller boats and PWC's (Figure 5.5).

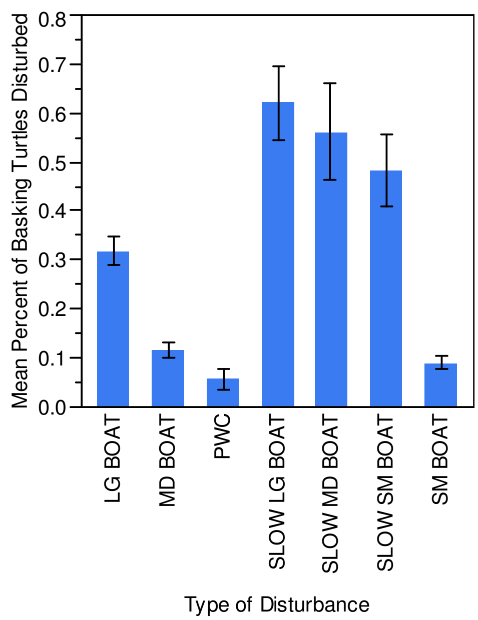


Figure 5.5. Mean percent of observed basking *G. flavimaculata* disturbed by type of recreational boat at the lower Pascagoula River site. Error bars represent one standard error.

Corticosterone Analysis.—We found that there was no difference in baseline corticosterone levels by site for either males ($X_2 = 0.87$, df = 1, $p = 0.35$; Leaf mean =

0.52 ng/mL, SD ± 0.69/ Pascagoula mean = 0.39 ng/mL, SD ± 0.51) or females (X_2 = 0.04, df = 1, p = 0.84; Leaf mean = 0.43 ng/mL, SD ± 0.64/ Pascagoula mean = 0.40 ng/mL, SD ± 0.69). However, we did find that there was a significant difference in stress response by sex ($F_{1,244}$ = 8.73, p = 0.003) with male stress response being significantly higher than female stress response (Figure 5.6); we did not find a difference in stress response by site ($F_{1,244}$ = 1.04, p = 0.31) or the sex by site interaction term ($F_{1,244}$ = 1.11, p = 0.29).

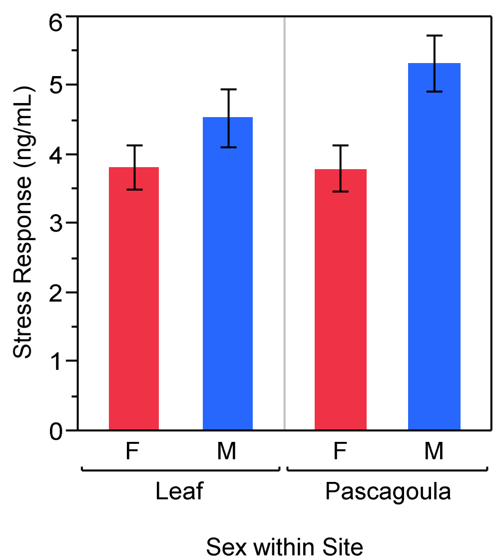


Figure 5.6. Mean stress response (ng/mL) by sex and site sampled. Error bars represent one standard error.

Shell Condition Analysis.—While examining captured turtles for the occurrence of shell fungus, we found that only 3.7% (7 of 187) of the turtles captured from the undisturbed Leaf River site had shell fungus present, whereas 26.1% (65 of 249) of the captured turtles from the Pascagoula site had signs of shell fungus. There was a significantly higher incidence of shell fungus among turtles from the Pascagoula River site in comparison to Leaf River turtles (X^2 = 45.1, df = 1, p = <0.001).

Analyzing Historical Boating Trends.—Over the last 22 years, the number of registered boats within Jackson County, MS has increased dramatically (by 300%) from 2033 registered boats in 1987/1988 to 6194 registered boats in 2007/2008 (Figure 5.7). Additionally, the period with the greatest 5-year increase was the most recent period, 2002/2003 to 2007/2008, with 4239 boats and 6194 boats registered during these periods, respectively (146% increase). The percentage of small boats (<16 ft) registered has also decreased from 57% of the total boats registered in 1987/1998 to 48% in 2007/2008. Conversely, the percentage of medium to large size boats (16-65 ft) registered has increased from 43% in 1987/1988 to 52% in 2007/2008.

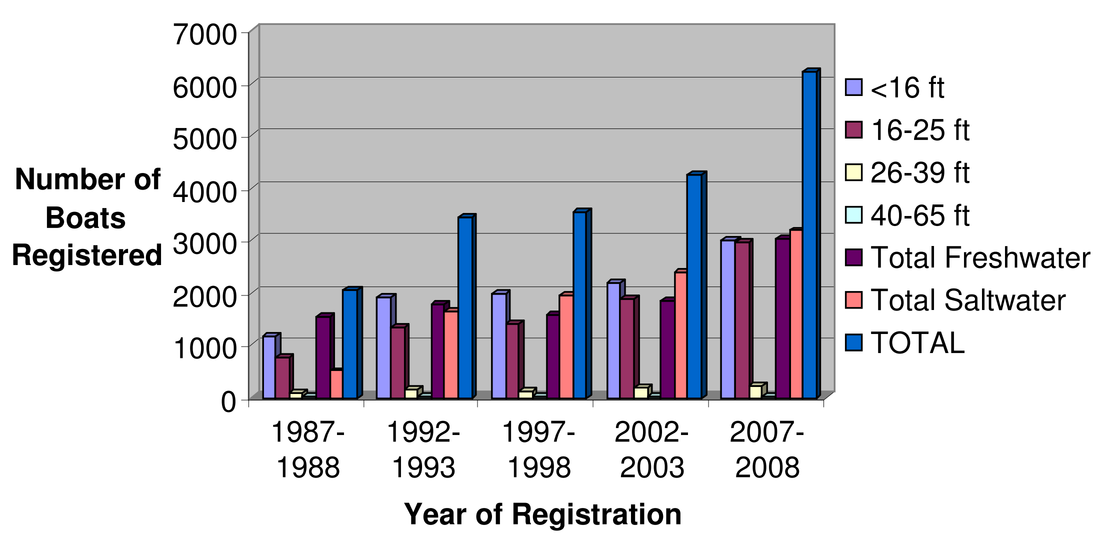


Figure 5.7. Number of boats registered in Jackson County, Mississippi (county of the Lower Pascagoula River site) over the last 22 years. Data provided by Gail Marshall of the Mississippi Department of Wildlife, Fisheries, and Parks.

DISCUSSION

*Impacts of Recreational Boating on Basking Behavior.—*We found that on a typical weekend and weekday at the Pascagoula River site, *G. flavimaculata* are approximately 22 times and five times more likely to be disturbed by human recreation in comparison to Leaf River turtles, respectively. Furthermore, undisturbed lower Pascagoula River turtles basked for longer durations than disturbed turtles from the lower Pascagoula River, while basking for similar durations relative to Leaf River turtles; this indicates that lower Pascagoula River turtles wish to achieve similar basking durations to Leaf River turtles, while also indicating that the thermal preferences remain similar across the two study sites. Overall, the observed differences in basking duration and basking percentages between the Leaf River site and the Pascagoula River site can be directly attributable to the increased amount of recreational boating that disturbs significantly more basking turtles from the Pascagoula River site. Additionally, basking durations of *G. flavimaculata* at the undisturbed Leaf River site were longer for almost every month and across every hour during the day in comparison to the Pascagoula River site, while the percentage of turtles basking also followed a similar pattern. Moore and Seigel (2006), however, found a bimodal basking frequency with *G. flavimaculata* in the Pascagoula River from turtles studied during the late 1990's, but this pattern did not exist for this study. This difference likely suggests that in the intervening time between the two studies, the number of recreational boaters has increased dramatically and this likely has impacted the basking frequency of turtles. Also, as documented by Moore and Seigel (2006), we found female *G. flavimaculata* were more likely to be disturbed than males.

Basking turtles were startled by the visual presence of the human disturbance, but were also often swept off their basking log by the boat wake that followed boat passage. Due to the number of individual turtles being monitored at a single time and the short time between the boat passage and the boat wake, this precluded us from deciphering the habituation of turtles to the human disturbance. However, qualitatively, turtles were habituated to boat passage at the Pascagoula River site in comparison to the turtles at the Leaf River site (W. Selman, pers. obs.). Also, a higher percentage of turtles were disturbed by slower boats (especially pontoon boats) relative to boats passing at a higher speed. Similarly, Moore and Seigel (2006) also found that *G. flavimaculata* were disturbed at a higher rate by slower moving boats, particularly fisherman setting trot-lines. Similarly, Burger (2001) found that Northern Watersnakes (*Nerodia sipedon*) and Common Gartersnakes (*Thamnophis sirtalis*) fled rapidly when humans stopped to watch snakes in comparison to walking past snake locations.

An increased number of human disturbances at the Pascagoula River site could likely increase energy expenditures by turtles that actively avoid the disturbance by diving into the water and swimming to safety, as well as turtles continuously having to pull themselves out of the water to bask after being disturbed. Also, one would expect turtle body temperatures to decrease after having to submerge following a disturbance, especially during the spring and fall months when water temperatures are much cooler than air temperatures (see Chapter IV). During the spring months, one would presume excess energy expenditure and decreased body temperatures would be particularly acute, especially for females during egg production, and could possibly account for the observed low clutch sizes, as well as the lowered clutch frequencies in comparison to upstream sites (Horne et al 2003, R. Vogt, pers. comm.).

Due to the unique life history of turtles, adult age classes are extremely sensitive to increased adult mortality or harvest (Congdon et al. 1993, 1994). During this study, several turtles showed evidence of direct impacts with boats, indicating that recreational boating in the lower Pascagoula River may pose an additional threat to turtle populations by direct mortality. This was not addressed in this study, but on several occasions dead, floating turtles were encountered that had been hit by a recreational boat, with evidence of successive propeller wounds. We presume, however, that most turtles that are hit by boats die and sink to the river bottom and are not readily seen. Therefore, turtle mortality from boat impacts is likely vastly undersampled. It is also probable that females are more likely to be hit by recreational boats since they are more commonly found in more open areas of the channel, away from the river bank (Jones 1996). Bulté et al. (2009) found that female northern map turtles (*Graptemys geographica*) were more likely to be hit by recreational boats and if only 10% of turtles that are hit by boats die, this could lead to localized population extinctions (Bulté et al. 2009). Therefore, recreational boating in the Pascagoula River may not only have a dramatic impact on turtle basking behavior, but also dramatic affects on turtle mortality and population declines.

Recreational boating also degrades nesting/river habitat due to excessive wake action. Several nesting beaches within in the Pascagoula River are protected for *G. flavimaculata* and have been significantly eroded by wake action (W. Selman, pers. obs.), with nesting beaches having a shelf-like or stair-step appearance. These vertical shelves might prevent nesting females from accessing desired nesting habitat.

Corticosterone Analysis.—Although frequent recreational disturbances occurred at the Pascagoula River site and adversely impacted basking behavior, we found that this had no impact on Pascagoula River turtle baseline corticosterone levels or the ability for turtles to mount a stress response relative to the mostly undisturbed Leaf River turtles. Currently, to our knowledge, there are no studies that examine both behavioral and physiological aspects of disturbance in turtles, but our findings are contrary to the few existing studies in birds/mammals. Most of these studies documented significant behavioral and/or corticosterone (corticoid) responses to human recreation. Thiel et al. (2008) documented that during ski-season in Germany, capercallie's (*Tetrao urogallus*) avoided areas of high ski activity within their home ranges and had larger home ranges during the non-ski season. They also found higher fecal corticosterone metabolites from birds inhabiting high traffic ski areas in comparison to low traffic areas. Furthermore, Walker et al. (2005) found that Megallanic penguin (*Spheniscus magellanicus*) chicks in tourist areas of Argentina did not flee when approached by humans and had higher stress responses in comparison to non-visited chicks. Further, adult penguins in tourist visited areas exhibited fewer head turns when approached by the researcher (habituation response to human presence) and had lower corticosterone levels following 30 minutes of restraint (Walker et al. 2006). The differences, especially given what is seen in birds and mammals, could relate to the fact that reptiles are heterothermic and therefore can not, or do not, significantly increase baseline levels of corticosterone for a prolonged period of time (although they can elevate corticosterone for short periods of time, see below).

It is unclear, however, why male *G. flavimaculata* had a higher stress response in comparison to females at both sites. It is possible that smaller males could perceive a stressful situation much differently than females since they are, on average, one-half the length and one-tenth the mass of females (Selman and Jones, 2009). A more likely explanation is that female stress response is considerably dampened during oviposition/ post-oviposition time period (Selman et al., unpubl. data) and this may account for the lower stress response that we found relative to males.

Shell Condition of G. flavimaculata.—The overwhelming difference of shell conditions between Leaf River turtles and Pascagoula turtles suggest that recreational disturbance is altering more than turtle basking times, but also impacting basic physiological processes like scute shedding. Basking during the morning and late afternoon appears critical for shell drying, particularly during the summer and early fall when laminal shedding occurs (W. Selman, pers. obs.). The Leaf River turtles consistently were able to bask for longer periods during the these times, whereas Pascagoula River turtles often abandoned basking during these times and would not reemerge readily; this could likely account for the increased levels of shell problems at the Pascagoula River site. In addition, few turtles at the Leaf River site had more than three fungal white spots present, whereas turtles from the Pascagoula River consistently had 10 or more present, with some having greater than 50% of the carapace covered. The shell of one female, for example, was found to be in poor condition and upon inspection by T. Majure (Former Aquarium Coordinator at Misssissippi Museum of Natural Science), he concluded that it looked similar to some captive *Graptemys* at the Mississippi Museum of Natural Science that were not able to bask under proper lighting conditions (Figure 5.8). The poor shell condition of the lower Pascagoula River turtles may also indicate poorer water quality relative to the Leaf River, but this was not addressed during this study and should be further pursued.

Analyzing Historical Boating Trends.—Boating registration data indicates that recreational disturbance within the lower Pascagoula River is a major concern with the number and size of boats increasing over the last 22 years; we encountered many local residents that also mentioned noticing this trend in the lower Pascagoula River. During the mid 1990's, B. Horne (pers. comm.) noted that while working in the Pascagoula River, a day could go by without seeing a boat and the vast majority of boats during this time period were local fishermen in small jon-boats. Conversely, 13 years later during this study, it was not uncommon to see 50 boats in a single day, with many of these being larger bass-fishing, off-shore, and recreational ski boats.

Figure 5.8. Captured female *Graptemys flavimaculata* from the Lower Pascagoula River site (4/30/08) that has widespread shell fungus.

Species Conservation and Recommendations.—Our data indicates that recreational disturbance is having a significant impact on the basking behavior of turtles within the Pascagoula River, both at the individual and population level, but this did not directly correlate with marked changes in turtle stress physiology. However, even though we did not document different baseline or stress response levels between the sites, we are cautious to presume that there are no physiological consequences for an altered basking behavior. First, we noted dramatically poorer shell condition from turtles from the Pascagoula River, indicating the inability for turtles to carry out the simple function of shedding carapacial scutes. Secondly, it is possible that there could be impaired physiological aspects that were not addressed by this study that could be impacted by excess recreational activity at the Pascagoula River site. These may include compromised female reproductive capacity and/or suppressed immune function/ability to fight infection. The former could be that females are not able to raise their body temperature to levels necessary to effectively yolk ovarian follicles (i.e. fewer eggs per clutch, smaller eggs, and/or fewer clutches) and the latter could be a lowered ability to fight-off infection since turtles likely cannot achieve their preferred body temperatures. Additionally, there may be significant impacts of recreational boating on direct turtle mortality. These topics, however, warrant future studies.

Based on our data, we recommend that there should be limited use of the river to small to medium size boats, since they disturb a significantly smaller percentage of basking turtles than larger boats. Boating restrictions should include preventing large boats (>20 feet long with deep draft hulls, including offshore and ski-boats) from accessing main river channels; larger boats also pose an erosion concern due to the large wakes that cause excessive erosion on turtle nesting sandbars and riverbanks. These regulations would likely improve the ability for turtles to bask, avoid excessive turtle mortality, and prevent nesting habitat erosion. Incidentally, if these regulations were enacted, they could also address a human safety concern due to the size of the wakes from larger boats that pose a risk for people operating smaller boats on the river.

CHAPTER VI

CONSERVATION GENETICS OF THE YELLOW-BLOTCHED SAWBACK (EMYDIDAE: *GRAPTEMYS FLAVIMACULATA*)

Abstract.—Population genetic data are becoming an important aspect of conservation planning in a variety of taxa. Turtles within the genus *Graptemys* are increasingly being recognized as a group needing conservation priority, especially since many are endemic to single Gulf of Mexico river drainages and vulnerable to human alterations of rivers/surrounding habitats. The Yellow-blotched sawback (*Graptemys flavimaculata*) is a federally Threatened, riverine turtle that is endemic to the Pascagoula River system, which is one of the last major free-flowing river systems in the lower United States. We used microsatellite data to analyze population genetic structure and assess the historical demography of six populations throughout the Pascagoula River system. Considerable allelic diversity was found in each of the populations and a demographic analysis failed to find evidence of recent or historical bottlenecks. Isolation by distance analysis indicated that genetic distance between sites, with the exception of the Escatawpa River site, correlated highly to geographic distance between sites. Analysis of molecular variance indicated that most of the variation was partitioned within rather than among populations, while STRUCTURE analysis indicated a single population (K = 1). Despite the relatively small contiguous river distances separating each population, most of the genetic comparisons among populations (as measured by F_{ST}) indicated a low, but significant degree of population structure, thus indicating a nonpanmictic population. Furthermore, most populations possessed one or more private alleles. If the habitat

preferred by *G. flavimaculata* is 'patchy' and discontinuous, then additional sampling in the headwaters of the Leaf River, in between our sampling localities, and in smaller tributaries may reveal additional low-level population structure.

INTRODUCTION

The intermingling of species conservation and genetics is becoming increasingly important with many habitats becoming fragmented/degraded by anthropogenic means or species being directly exploited by humans (e.g., for food or the pet trade). Conservation genetics provides a set of tools to assess the viability of individual populations, the connectivity among populations across various landscape scales, and the evolutionary uniqueness of certain lineages (Gibbs and Amato 2000). Of the 212 turtle/tortoise species assessed by the IUCN, 131 species (62%) are considered critically endangered, endangered, or threatened, with an additional eight species considered extinct or extinct in the wild (http://www.iucnredlist.org/about/summary-statistics, Table 4a; accessed 3 December 2009). However, to date, relatively few studies have taken a conservation genetic approach with freshwater turtles, even though freshwater turtles and tortoises are considered among the most endangered animal taxa in the world (IUCN 2008). Those studies that have investigated geographic patterns of population structure in freshwater aquatic turtles include studies of the western pond turtle (*Emys marmorata*- Gray 1995, Spinks and Shaffer 2005) and the painted turtle (*Chrysemys picta*- Starkey et al. 2003). Both studies detected considerable genetic variation over the entire species' ranges, as well as identifying critical populations subject to negative environmental impacts. However, both of these species have large ranges and individuals are known to move overland to other aquatic habitats (*E. marmorata*- Bury 1972, Holland 1992; *C. picta*- MacCulloch and Secoy 1983). To our knowledge, no research has focused on the population genetic structure of a riverine species that occurs exclusively within a single drainage, which includes the endemic *Graptemys* species of Gulf river drainages.

Turtles within the genus *Graptemys* (family Emydidae) are highly aquatic, freshwater turtles, with nine of twelve species endemic to single river drainages along the Gulf of Mexico in the southeastern United States (Ernst and Lovich 2009). Consequently, due to their restricted distributions and observed population declines, many of these turtles are also recognized as imperiled or in need of conservation (Ernst and Lovich 2009). *Graptemys*, like many other freshwater species, are extremely susceptible to human alterations of rivers and surrounding habitats, including river impoundments, channelization, water quality degradation via pollution/sedimentation, and operations that remove basking logs or snags (reviewed by Bulhmann and Gibbons 1997, Mitchell and Klemens 2000, Moll and Moll 2004).

The Yellow-blotched sawback (*Graptemys flavimaculata*) is a riverine turtle that is endemic to the Pascagoula River system of southern Mississippi, USA (Fig. 6.1), which is the least-impacted major river system in the lower United States (Dynesius and Nilsson 1994). *G. flavimaculata* occurs in the Pascagoula River and its tributaries, including the Leaf, Chickasawhay, and Escatawpa Rivers (Cliburn 1971; US Fish and Wildlife Service 1993; Selman and Qualls 2009b; Selman and Jones, 2010). Recently, populations were also detected in smaller drainages within the Pascagoula River system including portions of the Bouie River, Okatoma, Bluff, Tallahala, Bucatunna, and Bogue Homa creeks (Selman and Qualls 2009b). Observed population declines of *G.*

flavimaculata led to Federal listing as a threatened species in 1991 (US Fish and Wildlife Service 1991). In addition, following Hurricane Katrina, lower Pascagoula River populations were negatively affected and the population declined 47% (Selman and Qualls 2008), presumably due to a loss or eradication of prey items due to decreased water quality (increased salinity, decreased dissolved oxygen).

Since there are many factors, both anthropogenic and natural, that negatively impact *Graptemys* populations, it is critical to continue research on a range-wide level, including the movements of turtles between populations. For example, these turtles are not equally abundant throughout their range; their distribution is noticeably ‘patchy’, with few or no individuals present in some stretches of the river system (Selman and Qualls 2009b). This raises the following questions: 1) Is there population structure within the range of *G. flavimaculata*? 2) If so, what does this tell us about the ecology/life history of the species? 3) How does this apply to the current/future species management plans?

MATERIALS AND METHODS

Sampling.—From 2005-2009, we collected tissue/blood samples at several sites within the Pascagoula River basin of southeastern Mississippi including: (1) upper Leaf River (north of Hattiesburg, Forrest County), (2) lower Leaf River (near Beaumont, Perry County), (3) upper Chickasawhay River (south of Stonewall, Clarke County), (4) lower Chickasawhay River (near Leakesville, Greene County), (5) Lower Pascagoula River (near Vancleave, Jackson County), and (6) Escatawpa River (north of Orange Grove, Jackson County, Figure 6.1). Turtles were trapped by submerging basking traps (made of

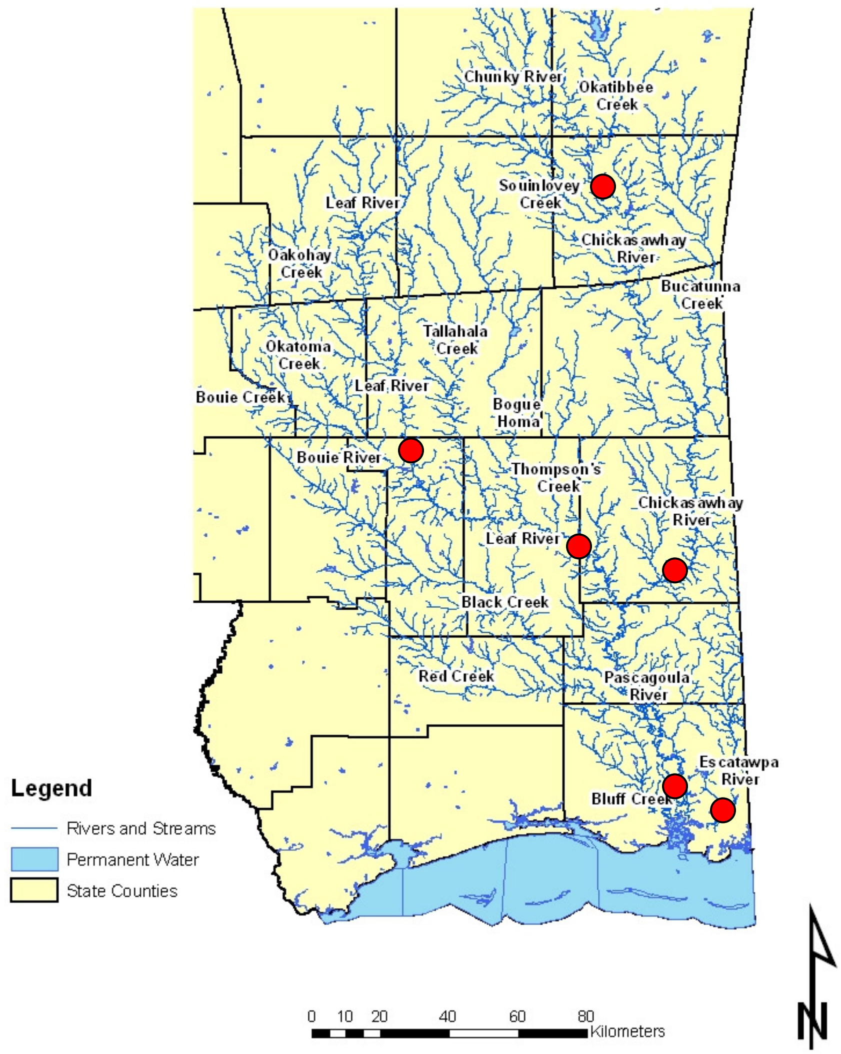


Figure 6.1. Population genetics sampling sites (circles) for *G. flavimaculata* within the Pascagoula River system of southeastern Mississippi, USA.

¾" PVC coated crawfish wire) from observed turtle basking structures and also captured opportunistically by dip net while turtles were basking on emergent deadwood or swimming near the water surface. For specific information on capture techniques see Selman and Qualls (2009b).

Once captured, tissue samples were collected by one of two methods. Blood was collected from individuals from the coccygeal vein (tail) using a heparinized 1mL syringe and a 26 ½ gauge needles. Blood samples (appox. 0.75-1 mL) were stored on ice for 4 to 6 hours and then centrifuged. Plasma and blood cells were then separated, and were then frozen. Alternatively, tails tips were collected and stored in 100% ethanol. For each captured individual, locality data was collected via GPS (Garmin GPS 72), as well as other information including sex and morphometrics for a related study.

Microsatellite Analysis.—Microsatellite loci have not been isolated for *G. flavimaculata*, but given the potential for cross-species amplification of microsatellite loci among related species (e.g. FitzSimmons et al. 1995, Primmer et al. 1996, Rico et al. 1996, Holman et al. 2005), we were able to use six microsatellite loci originally developed for other related Emydid turtles (*Malaclemys terrapin*- *TerpSH2*, *TerpSH5*, *TerpSH7* [Hauswaldt and Glenn 2003]; *Glyptemys muhlenbergii*- *GmuB08*, *GmuD70*, *GmuD88* [King and Julian 2004]). We found these loci to be polymorphic and cross-amplify reliably for *G. flavimaculata* (Selman et al. 2009b). Total genomic DNA was extracted from individuals from each site with the DNeasy Tissue Kit (QIAGEN Inc.). Although heparin can sometimes have an inhibitory effect on the polymerase chain reaction (Beutler et al. 1990), this extraction method yielded good quality DNA that amplified well for most individuals. Amplifications were conducted in a total volume of either 12.5 µl or 25 µl (depending on locus used) using 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.01% gelatin, 200 µM dNTPs, 2 mM $MgCl_2$ (4 mM for *TerpSH7*), 0.5 units of *Taq* polymerase (Promega Co.), 0.3 µM of the M13 tailed forward primer (Boutin-Ganache et al. 2001), 0.3 µM of the reverse primer, 0.1 µM of the labeled M13 primer (LICOR Co.), 20-150 ng of template DNA, and water to the final volume. PCR cycling conditions consisted of an initial denaturing step of 94°C for 2 min followed by 35 cycles of 30 sec at 94°C, 1 min at 52-60°C, and 1 min at 72°C. A final elongation step of 10 min at 72°C ended the cycle. Microsatellite alleles were visualized on acrylamide gels using a LICOR 4300 DNA analyzer and scored using Gene Image IR v. 3.55 (LICOR Co.).

Statistical analysis.—Genetic variation in each *G. flavimaculata* population was described using standard measures, including number of alleles (N_a), effective number of alleles (N_e), number of private alleles (N_{pa}), observed heterozygosity (H_o), and expected heterozygosity (H_e) as calculated by GenAlex 6.1 (Peakall et al. 2006). Loci were tested for Hardy-Weinburg equilibrium and linkage disequilibrium using GENEPOP for the web (Raymond and Rousset 1995; http://genepop.curtin.edu.au/). Program FSTAT 2.9.3 (Goudet 1995) was used to determine genetic differentiation among populations (F_{ST}), for significance testing of F_{ST} values, and to determine rarified number of alleles (A_R).

We also used several other methods to determine if population structure existed between collection localities for *G. flavimaculata*. STRUCTURE 2.3.1 (Pritchard et al. 2000) uses a Bayesian approach to estimate the likelihood of individuals being assigned to different populations in such a way that the populations are in Hardy-Weinberg equilibrium and the loci in each population are in linkage equilibrium. We tested values of *K* (number of populations) from 1-8 using a model of admixed ancestry and assuming independent allele frequencies between groups. For each value of *K* we performed 10 separate simulations with a burn-in of 50,000 generations followed by a subsequent 100,000 generations. Furthermore, an analysis of molecular variance (AMOVA; Excoffier et al. 1992) as implemented by ARLEQUIN v. 3.11 (Excoffier et al. 2005) was used to examine the distribution of molecular variation within and among populations. Lastly, we used a Mantel test using the Isolation By Distance Web Service (IBDWS;

Jenson et al. 2005) to determine if genetic differentiation was correlated to geographic distance between sampling localities (regression of F_{ST} values and geographic distance values [in river kilometers]).

Another important way that genetic data can be analyzed in a conservation context is by distinguishing populations with reduced levels of genetic variability as a function of historically small populations versus populations that have recently experienced a population bottleneck. During a bottleneck, allelic diversity is lost faster than population heterozygosity, with the observed number of alleles being less than the number expected from observed Hardy–Weinberg heterozygosity (Cornuet and Luikart 1996); recent population bottlenecks may be detected using program BOTTLENECK ver. 1.2 (Piry et al. 1999). A two-phase mutation model (TPM) was used with a 95% proportion of stepwise mutation model, 10% variance, and run with 1000 iterations (e.g. Di Rienzo et al. 1994, Piry et al. 1999, Jones et al. 2004) with all six sampled populations. To detect a population bottleneck, we thereafter used a one-tailed, Wilcoxon Sign Rank Test to detect heterozygosity excess in comparison to expected heterozygosity at equillibrium.

RESULTS

A total of 241 individuals were genotyped from the six sample localities and across the six microsatellite loci. This included genotyping 60 individuals from the lower Chickasawhay, 54 from the Pascagoula, 52 from the upper Leaf, 32 from the Escatawpa, 22 from the lower Leaf, and 21 from the upper Chickasawhay. None of the populations deviated from Hardy-Weinberg equilibrium expectations and none of the loci demonstrated linkage disequilibrium after sequential Bonferroni corrections (Rice 1989). Patterns of genetic diversity within populations (allele size, sample site, number of individuals genotyped at locus [*N*], number of alleles [*A*], effective number of alleles [N_e], allelic richnes [A_R], observed heterozygosity [H_O], expected heterozygosity [H_E], and private alleles in bold) are shown for all populations at each locus in Appendix B.

Among-population differentiation.—Pairwise F_{ST} values were all relatively small, but were highest between the Escatawpa/Upper Leaf (F_{ST} = 0.0473, 256.7 river km; Table 6.1). The least differentiation observed was between the lower Chickasawhay/ Pascagouala sites (F_{ST} = 0.0058, 144.9 river km). In general, the highest F_{ST} values were associated with comparisons that included the Escatawpa site. The likelihood scores from STRUCTURE indicated that a K of 1, or one population, was the best model of population structure. The average log likelihood for K of 1 was -5384.3 (SD =0.39), which was the least negative value among the different values of K. Similarly, the results of the rangewide AMOVA indicated that most of the genetic variation was partitioned within populations (97.84%) rather than among populations (2.16%), although the global F_{ST} value (0.0126) was significant (p<0.0001). Results from our isolation by distance test indicated that there was an insignificant correlation of genetic distance and river distance

Table 6.1. Pairwise F_{ST} values (below diagonal) and geographic distances (above diagonal; in river kilometers) among 6 sampling sites for *G. flavimaculata* within the Pascagoula River system. * Denotes F_{ST} values that deviated significantly from zero.

	Pascagoula	Lower Chickasawhay	Upper Leaf	Escatawpa	Upper Chickasawhay	Lower Leaf
Pascagoula	-	144.9	222.4	34.3	346.4	145.2
Lower Chickasawhay	0.0058*	-	165.1	179.2	201.5	87.9
Upper Leaf	0.0142*	0.0122*	-	256.7	366.6	77.2
Escatawpa	0.0364*	0.0434*	0.0473*	-	380.7	179.5
Upper Chickasawhay	0.0209*	0.0212*	0.0405*	0.0388*	-	289.4
Lower Leaf	0.0156*	0.0130	0.0153*	0.0301*	0.0335*	-

for our six study sites (Z = 88.0712, R^2 = 0.5714, p = 0.0750; Figure 6.2). However, this pattern appeared to be driven by the higher than expected differences between the Escatawpa versus other sites (discussed below). Indeed, this correlation was significant, when the Escatawpa site comparisons were removed from the analysis (Z = 44.7094, R^2 = 0.9099, $p < 0.001$; Figure 6.3).

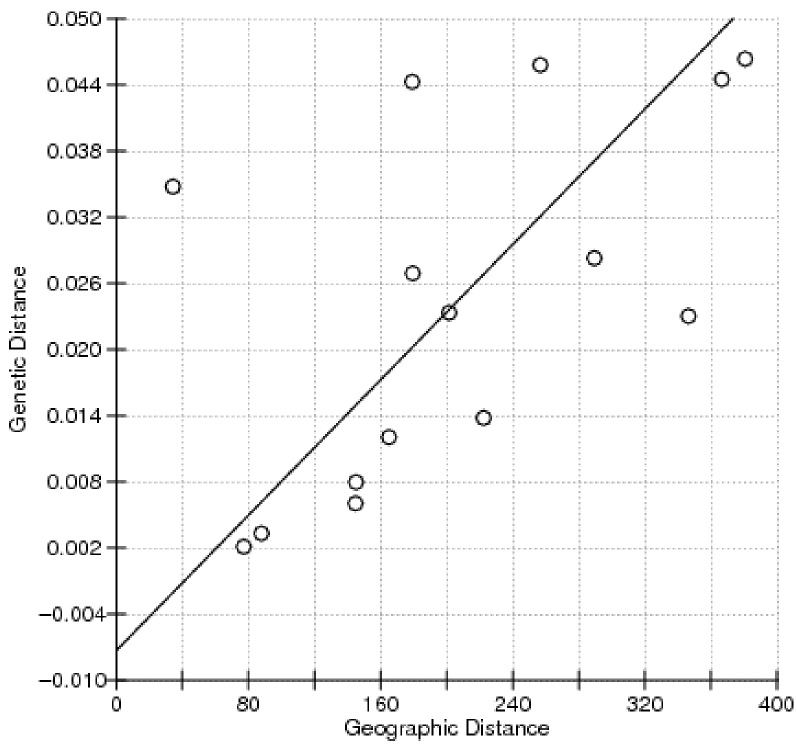


Figure 6.2. Results from the Mantel test to determine isolation by distance among our six sampled populations (Z = 88.0712, R^2 = 0.5714, p = 0.0750). Genetic distance was calculated as: F_{ST} / (1- F_{ST}).

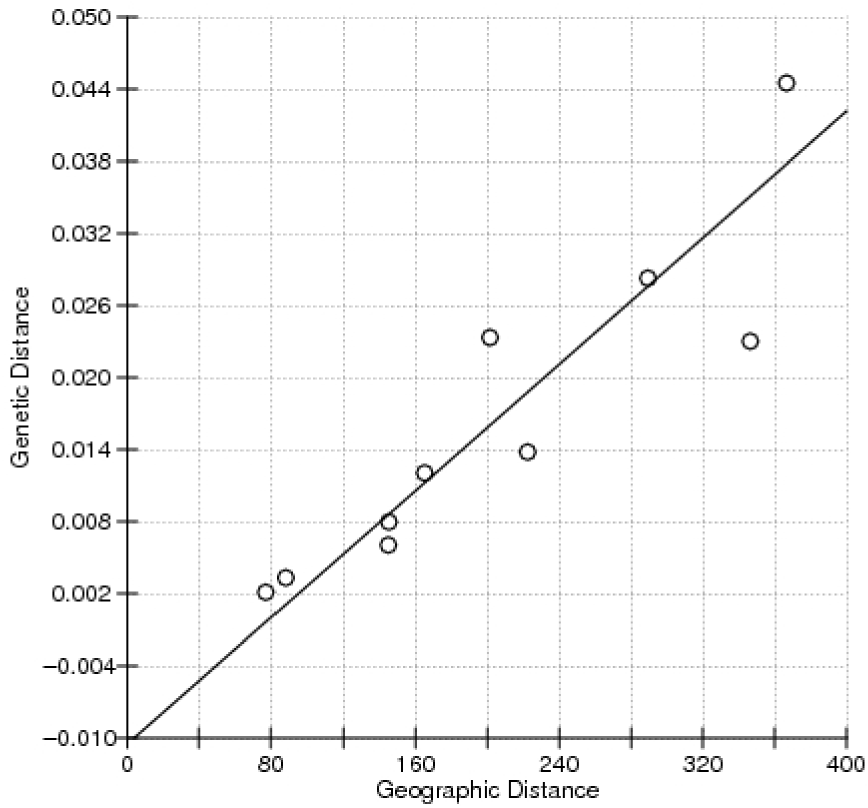


Figure 6.3. Results from the Mantel test to determine isolation by distance at five sampled populations, excluding the Escatawpa River site (Z = 44.7094, R^2 = 0.9099, p < 0.001). Genetic distance was calculated as: F_{ST} / (1- F_{ST}).

Was there a historical population bottleneck?—Historical demographic tests using BOTTLENECK did not find any evidence of a recent or historical population bottleneck. The one-tailed test for heterozygosity excess in BOTTLENECK was not significant (p >0.05) for all populations except the lower Pascagoula River site (p = 0.039); this value was not significant, however, after sequential Bonferroni correction (Rice 1989).

DISCUSSION

Population Structure.—Our analyses indicate that there is little genetic structure between sampling localities as suggested by AMOVA and the program STRUCTURE; these analyses indicated a high level of genetic variation within populations rather than among populations and an optimal K value of 1, respectively. Genetic distance (F_{ST}) within the main river populations (excluding Escatawpa River population) appears to correlate well to geographic distance between sampling sites. Generally, sites that were closer to each other had smaller F_{ST} values, while sites that were farther away from each other had larger F_{ST} values, which would be expected in a continuous river system with individuals with relatively small, established home ranges (Jones 1996). While there are not many examples for comparison in the literature, this relationship was not similar in the Giant Amazon River turtle, *Podocnemis expansa* (Pearse et al. 2006); when comparing populations within river basins, they found that only 2 of the 11 pairwise F_{ST} values were significantly different from zero, while all were significant for interdrainage comparisons. They concluded that extensive gene flow was occurring within drainages and little gene flow was occurring among drainages. This is likely due to the high vagility of *P. expansa* and the large seasonal movements that are associated with moving to feeding or nesting grounds (von Hildebrand et al. 1997).

Our results also seem to contradict the idea that there may be higher allelic diversity in downstream populations due to turtles that might be forced downstream during high water events. If this were the case, one would predict that there should be a greater degree of population differentiation and more private alleles for the upstream populations, while genetic variability in the downriver population would be a mixture of the upstream sources. F_{ST} values and the presence of private alleles do not support this prediction of upstream mixture within the lower Pascagoula River population.

The question we cannot answer with this study is the measure of genetic divergence of headwater populations that were not sampled (i.e., above upper Leaf River site), populations between sampling sites (i.e., 201.5 river km between upper and lower Chickasawhay River sampling sites), and smaller tributary populations (i.e., Tallahala, Creek). Sampling for individuals at additional sites in these reaches was difficult and not possible in some areas due to decreased river accessibility due to 1) lack of public boat ramps, 2) poor channel navigability, and 3) difficulty in capturing low density populations of *G. flavimaculata*. The known range of *G. flavimaculata* within the Leaf River extends 98 river km upstream of our upper Leaf River site, while our upper Chickasawhay River site was within 10 km of the upstream distributional limit in that tributary. Within these northern stretches of the Leaf and Chickasawhay rivers and also within the smaller tributaries, *G. flavimaculata* exhibits a very ‘patchy’ distribution with individuals apparently occupying only patches of medium to high quality habitat (i.e. open canopy for better basking conditions, higher density of basking snags, moderate flow, presence of nesting sandbars). These patches of good habitat are often separated by several kilometers of apparently marginal/unsuitable habitat (i.e., closed canopy, few snags, high flow with rapids, no sandbars) and therefore, it becomes unfavorable for turtles to venture to the next patch of suitable habitat (Shively and Jackson 1985). The movement of *G. flavimaculata* in these more northerly reaches of the river system is unknown, especially with respect to movement of individuals among these favorable habitat patches. Within a more homogenous environment of the lower Pascagoula River, average linear home ranges for *G. flavimaculata* vary from less than 200m to as much as 6 km (Jones 1996). Therefore, the ability of these turtles to move long, linear distances is possible, but it is unknown if individuals in these northern reaches will inhabit different patches of favorable habitat by moving long distances through unfavorable habitat.

Was there a historical population bottleneck?—Our analyses indicate that there is little evidence for a recent or a historical genetic bottleneck for any of the sampled *G. flavimaculata* populations, even though there were observed range-wide population declines in the 1980's (McCoy and Vogt 1980, Stewart 1989). Most turtle species that exhibit a population/genetic bottleneck are likely to have been subject to high levels of human harvest, including *Podocnemis expansa* (Pearse et al. 2006). There are no historical records that indicate that *G. flavimaculata* has been the subject of high historical or recent human harvest/consumption, likely due to the smaller size of *G. flavimaculata* in comparison to other larger species inhabiting the Pascagoula River. Therefore, we presume that these larger turtle species may have historically been better suited to provide an adequate protein source relative to *G. flavimaculata* (including the river cooter- *Pseudemys concinna*; common slider- *Trachemys scripta*; common snapping turtle- *Chelydra serpentina*; alligator snapping turtle- *Macrochelys temminckii*; and softshell turtles- *Apalone sp.*).

Significance of Escatawpa Population.—Genetically, *G. flavimaculata* from the Escatawpa River are the most distinct among the six sites we sampled. This is evident by the Pascagoula River site/Escatawpa River site comparison which had one of the highest pairwise F_{ST} value (F_{ST} = 0.0364), while it had the smallest geographic distance of all sites tested (34.3 river km). In comparison, the geographic distance between the upper

Leaf River/upper Chickasawhay River is ten-fold longer (366.6 river km), but had a similar F_{ST} value ($F_{ST} = 0.0405$). Furthermore, the Escatawpa River site deviated more than any other site in our isolation by distance model and although small, contributed to most of the genetic variation among sites within our AMOVA analysis.

Currently, unsuitable brackish marsh habitat exists in most of the 34.3 river km between the lower Pascagoula River and Escatawpa River sites. Historically, Otvos (*in press*) suggested that the Escatawpa River flowed into Grand Bay (approx. 16.5 km east of the Pascagoula River mouth) and was only recently captured by the Pascagoula River (within the last 11,500 years; Figure 6.4). We suspect that the deeper genetic separation of the Escatawpa River population found by this study is more likely attributable to the historical geographic separation of the Escatawpa River relative to the Pascagoula River, as suggested by Otvos.

Currently, the extent of gene flow between the lower Pascagoula and Escatawpa River sites is unknown, but we suspect it is minimal to non-existent; this, however, warrants future studies. This population is also considered one of the most endangered populations of *G. flavimaculata* (Selman and Jones, 2010) and this population likely consists of fewer than 500 individuals (W. Selman, pers. obs.).

Conclusions.—The finding of little population structure does not suggest that there is little need for conservation of *G. flavimaculata* and riverine habitat throughout the drainage. The significant F_{ST} values between all population comparisons except one, and the presence of private alleles in populations, indicate that *G. flavimaculata* does not represent a panmictic population throughout the Pascagoula River system, as would be

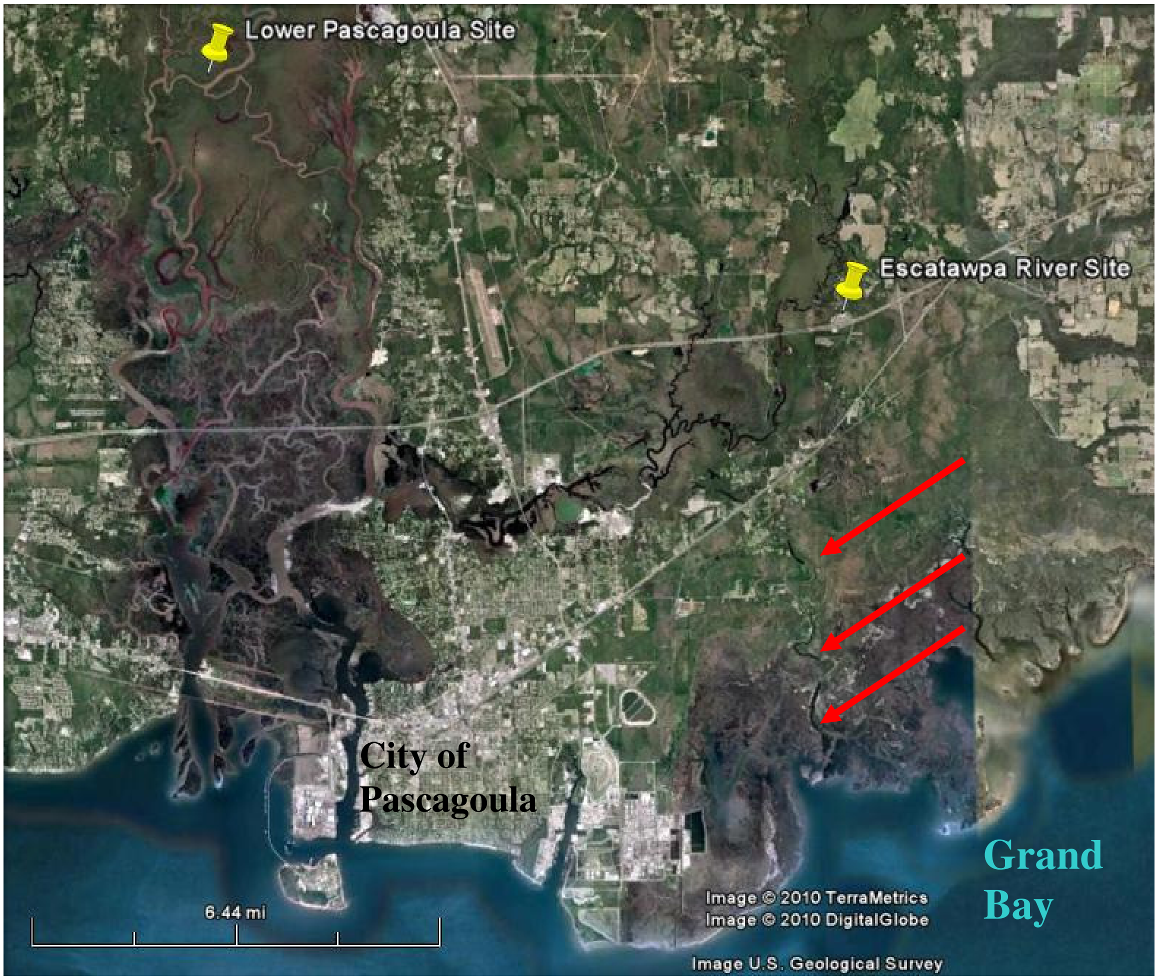


Figure 6.4. Google Earth image of the lower Pascagoula River and Escatawpa River sites. Relict channel of the Escatawpa River that historically flowed into Grand Bay (indicated by arrows), as proposed by Otvos (*in press*).

expected in a continuous river system. Due to the small size of the Escatawpa population, the higher levels of genetic differentiation, and the unique geological history of the Escatawpa river system, this population should be further studied to determine the extent of gene flow to/from the main Pascagoula River populations, as well as further determine the population status. Future management of this species should take into account the differences among populations, particularly if reintroductions are needed in the future for this federally Threatened species.

CHAPTER VII

GENERAL CONCLUSION AND RECOMMENDATIONS

Our research supports several conservation measures that will benefit the Yellow-blotched sawback, and in general, other Pascagoula River turtles and aquatic fauna. We propose that state and federal managers, and to some level, private entities can improve the suitability of occupied rivers/creeks, by minimizing habitat alteration (i.e. de-snagging projects, impoundments, channelization), promoting streamside management zones and proper forest management practices, controlling invasive species, and providing an education program related to the Pascagoula River system and endangered species (with emphasis on turtles).

Specifically, incentive programs should be offered to landowners within the Pascagoula River system that live adjacent to rivers where high densities of *Graptemys flavimaculata* occur. These agreements should specifically address the maintenance of streamside management zones which will greatly improve riparian edges and *G. flavmaculata* habitat. In addition, the invasive species cogongrass (*Imperata cylindrica*) appears to be a growing concern due to the dispersal ability of this species, as well as the ability to alter nest temperatures and nest locations of *G. flavimaculata* (Horne 1999). Much has been done on state managed properties for cogon grass removal, but there is currently no incentive for private landowners to remove cogon grass from their riverbanks or sandbars. Without private landowner's cooperation, future cogon grass removal on state/federal managed sandbars will be a continuous and costly effort. Also, subsidized predators (feral swine [*Sus scrofa*], raccoons [*Procyon lotor*], and fish crows [*Corvus ossifragus*]) need to be controlled to prevent excessive nest predation. Lastly, continued educational outreach is needed for children, as well as adults, on the importance of turtles to the river ecosystem. Much effort has been devoted to the education of children, but some form of adult education is needed due to the lack of knowledge by many adults on the existence of this river turtle and the value of it to the river system. Many locals, particularly fisherman, have been encountered during the course of this study. These fishermen had fished certain parts of the river for their whole lives and they had no idea that 1) there is an endangered river turtle that inhabits only the Pascagoula River system, 2) this turtle is a unique species, and 3) the benefit of turtles to the river system. Almost all people encountered were interested and held neutral to positive attitudes after they were informed about the turtle.

Next, appropriate regulatory activities should include increasing law enforcement to curtail illegal collection, shooting of turtles, and ATV use on nesting sandbars, while also enacting proper legislation to limit the recreational use of the lower Pascagoula River to small or medium sized boats. Suspending recreational boating throughout the Pascagoula River is highly unlikely. Therefore, we propose that there should be boating restrictions via new boating legislation to prevent large boats (>20 feet with deep draft hulls) from accessing main river channels and should be limited to only open water use (i.e., use in reservoirs or coastal areas). These larger boats disturbed a higher percentage of turtles than any other type of boat/watercraft. This could also address not only the impact on basking turtles, but also address a safety concern due to the size of the wakes that affect other smaller watercraft that use the river. Larger boats also pose an erosion concern due to the large wakes that cause excessive erosion on sandbars and banks.

Future areas that should be protected throughout the Pascagoula River system include properties bordering the lower Escatawpa River due to the small size and genetically distinct population of *Graptemys flavimaculata*, as well high turtle density areas within the Leaf and Chickasawhay rivers. The latter should include conserving property north of Hattiesburg (Forrest Co.) on the Leaf River since this area supports the northernmost substantial population of *G. flavimaculata*, as well as a sizeable population of *G. gibbonsi*. Similarly, property south of Stonewall (Clarke Co.) on the Chickasawhay River should be conserved due to sizeable populations of both *Graptemys* species. Equally important to proper riparian habitat protection, is maintaining the free-flowing nature of the Pascagoula River which will preserve the connectivity of riverine habitat and the unique yearly river cycles that are so important to aquatic fauna. If reservoirs are constructed and make a non free-flowing environment, *Graptemys* turtles will likely be outcompeted by lentic turtle generalists (i.e., Red-Eared Slider [*Trachemys scripta elegans*]) and will also lose connectivity with upstream or downstream populations.

Future research should include regularly assessing marked populations at established sites (Hattiesburg, Leakesville, Vancleave, Beaumont, Stonewall, and Escatawpa) to determine population density/viability, demography, growth, long-term movements, and longevity of *G. flavimaculata*. Additionally, future work should determine the size of the Escatawpa population, as well as the current connectivity/isolation to the Pascagoula River population. Since little is known about smaller populations from smaller, headwater rivers/creeks, research should extend to these populations to determine the viability of these populations, movement of individuals among habitat patches, and the general ecology of upriver populations.

For this research we used a wide array of research methods/tools (population surveys, basking density surveys, molecular/endocrinological techniques) to provide the information necessary to determine the conservation status and ecology of *Graptemys flavimaculata*. We hope that the information gathered during this study will provide quality information for conservation planners/managers to preserve viable turtle populations and intact riverine habitat throughout the Pascagoula River system into the future.

APPENDIX A

BRIDGE SURVEY LOCALITIES WITHIN THE PASCAGOULA RIVER SYSTEM, MISSISSIPPI, USA

Site	Location	County	Nearest Town (Direction)
1	Chickasawhay Cr., MS Hwy 495	Kemper	Prismatic (E)
2	Okatibbee Cr., Frazier Grove Rd.	Lauderdale	Martin (W)
3	Okatibbee Cr., State Blvd. Extension	Lauderdale	Meridian (E)
4	Okatibbee Cr., MS Hwy 19	Lauderdale	Meridian (E)
5	Okatibbee Cr, Arundel Rd.	Lauderdale	Arundel (W)
6	Okatibbee Cr, Bronson Rd Bridge	Lauderdale	Savoy (W)
7	Chunky Cr., US Hwy 80	Newton	Hickory (W)
8	Chunky Cr., US Hwy 80	Newton	Chunky (W)
9	Chunky Cr., US Hwy 80	Lauderdale	Chunky (W)
10	Tallahatta Cr., US Hwy 80	Lauderdale	Meehan (E)
11	Chunky Cr., Stuckey Bridge Rd.	Lauderdale	Meehan (N)
12	Chickasawhay River, MS Hwy 513	Clarke	Enterprise (W)
13	Chickasawhay River, River Road	Clarke	Stonewall (E)
14	Chickasawhay River, MS Hwy 512	Clarke	Quitman (E)
15	Souinlovey Cr., MS Hwy 512	Clarke	Pachuta (W)
16	Souinlovey Cr., Clarke Co. 120/ Harmony-Elwood Rd	Clarke	Harmony (S)
17	Chickasawhay River, US Hwy 45 & US Hwy145	Clarke	Quitman (N)
18	Chickasawhay River, US Hwy 145	Clarke	Shubuta (N)
19	Chickasawhay River, US Hwy 45	Wayne	Shubuta (N)
20	Chickasawhay River, US Hwy 84 & US Hwy 184	Wayne	Waynesboro (E)
21	Chickasawhay River, MS Hwy 63	Wayne	Waynesboro (N)
22	Chickasawhay River, Buckatunna-Chicora-Clara Rd	Wayne	Buckatunna (E)
23	Bucatunna Creek, US Hwy 84	Wayne	Gretna (E)
24	Bucatunna Creek, Denham- Winchester Rd	Wayne	Denham (W)
25	Bucatunna Creek, Buckatunna-Millry Rd	Wayne	Buckatunna (W)
26	Bucatunna Creek, US Hwy 45	Wayne	Battles (S)
27	Chickasawhay River, MS Hwy 42	Greene	State Line (E)
28	Chickasawhay River, Martin Luther King Dr.	Greene	Old Avera (W)
29	Chickasawhay River, Hwy 57	Greene	Leakesville (W)
30	Big Cr., Hwy 57	Greene	Leakesville (E)
31	Big Cr., St. Ellen Rd.	Greene	Leakesville (NE)
32	Chickasawhay River; Hwy 98	Greene	Merrill (S)
33	Leaf River, Smith Co. Rd. 126	Smith	Raleigh (SW)
34	Leaf River, Hwy 18	Smith	Sylvarena (E)
35	Leaf River; Smith Co. Rd. 84	Smith	Center Ridge (W)
36	West Tallahala Cr., Hwy 18	Smith	Sylvarena (W)
37	Leaf River; Smith Co. Rd. 80	Smith	Center Ridge (W)
38	Leaf River; Hwy 28	Smith	Taylorsville (W)
39	Oakohay Cr., Hwy 18	Smith	Raleigh (E)
40	Oakohay Cr., Hwy 540	Smith	Raleigh (E)
41	Oakohay Cr., Hwy 28	Smith	Mize (W)
42	Oakohay Cr., Smith Co. Rd. 14	Smith	Taylorsville (E)
43	Oakohay Cr., Hwy 37	Covington	Hot Coffee (W)

Site	Location	County	Nearest Town (Direction)
44	Leaf River, Hwy 84	Covington	Hot Coffee (N)
45	Big Creek, Sandhill Church Rd.	Jones	Ellisville (E)
46	Leaf River; Hwy 588	Jones	Ellisville (E)
47	Leaf River, Hwy 590	Jones	Moselle (S)
48	Leaf River, Church St.	Jones	Eastabuchie (W)
49	Bouie Cr., Hwy 541	Jefferson Davis	Clem (SW)
50	Bouie Cr., Ramsey Rd.	Jefferson Davis	Mt. Carmel (S)
51	Bouie Cr., Hwy 84	Covington	Lonestar (E)
52	Bouie Cr., Hwy 35	Covington	Lonestar (N)
53	Bouie Cr., Lonestar Rd.	Covington/ Jefferson Davis	McRaney (E)
54	Bouie Cr., Wilson Rd	Covington/ Jefferson Davis	Williamsburg (N)
55	Bouie Cr., Williamsburg Rd.	Covington/ Jefferson Davis	Williamsburg (N)
56	Bouie Cr., Oakdale Church Rd/Crossroad Ch. Rd	Covington/ Jefferson Davis	Seminary (E)
57	Bouie Cr., Seminary- Sumrall Rd	Covington	Sumrall (S)
58	Bouie Cr., Hwy 589	Covington	Sumrall (S)
59	Okatoma Cr., Hwy 35	Covington	Mt. Olive (W)
60	Okatoma Cr., Hwy 84	Covington	Collins (W)
61	Okatoma Cr., Hwy 590	Covington	Seminary (E)
62	Okatoma Cr., Hwy 598	Covington	Sanford (E)
63	Okatoma Cr., Lux Rd	Covington	Lux (E)
64	Bouie River, Glendale Rd.	Forrest	Hattiesburg (S)
65	Bouie River/ Leaf River, Hwy 42	Forrest	Hattiesburg (S)
66	Leaf River, East Hardy St.	Forrest	Hattiesburg (W)
67	Leaf River, Sims Rd.	Forrest	Hattiesburg (N)
68	Tallahala Cr., Hwy 15	Jones	Laurel (N)
69	Tallahala Cr., Luther Hill Rd.	Jones	Laurel (N)
70	Tallahala Cr., Paulding Rd.	Jones	Ellisville (W)
71	Tallahala Cr., Hwy 29	Jones	Ellisville (W)
72	Tallahala Cr., Three Mile Stretch/ Augusta Rd.	Jones	Ellisville (N)
73	Tallahala Cr., Ovett- Moselle Rd.	Jones	Union (W)
74	Tallahala Cr., Morriston Rd.	Forrest	Runnelstown (S)
75	Tallahala Cr., Hwy 42	Perry	Runnelstown (E)
76	Tallahala Cr., MS Hwy 42	Perry	Runnelstown (E)
77	Tallahala Cr., Thomas Creek Rd.	Perry	Runnelstown (N)
78	Tallahala Cr., Old River Rd.	Perry	New Augusta (E)
79	Leaf River, Hwy 29	Perry	New Augusta (E)
80	Bogue Homa Cr., Hwy 15	Jones	Laurel (N)
81	Bogue Homa Cr., Old Hwy 15	Jones	Ovett (S)
82	Bogue Homa Cr., Ovett- Moselle Rd.	Jones	Ovett (E)
83	Bogue Homa Cr., Whitfield Rd.	Perry	Whitfield (NW)
84	Bogue Homa Cr., MS Hwy 42	Perry	Richton (E)
85	Bogue Homa Cr., Old Augusta Rd.	Perry	New Augusta (S)
86	Leaf River, Wingate Rd.	Perry	New Augusta (W)
87	Leaf River, Hwy 15	Perry	Beaumont (S)
88	Thompson Cr., Hintonville Rd.	Perry	Hintonville (W)
89	Thompson Cr., Arlington Rd.	Perry	Beaumont (W)
90	Leaf River, Old Hwy 24	Greene	McLain (S)
91	Leaf River, Hwy 98	Greene	McLain (W)
92	Pascagoula River, Main St. Merrill Bridge	George	Merrill (E)

Site	Location	County	Nearest Town (Direction)
93	Pascagoula River, Hwy 26	George	Benndale (W)
94	Black Cr., Old Hwy 24	Lamar	Hattiesburg (E)
95	Black Cr., Hwy 589	Lamar	Hattiesburg (E)
96	Black Cr., Old Hwy 11	Lamar	Purvis (S)
97	Black Cr., Browns Bridge Rd.	Forrest	Purvis (W)
98	Black Cr., Churchwell Rd.	Forrest	Rock Hill (S)
99	Little Black Cr., Rock Hill- Carnes Rd.	Forrest	Rock Hill (S)
100	Black Cr., Old Hwy 49	Forrest	Brooklyn (N)
101	Black Cr., Ashe Nursery Rd.	Forrest	Brooklyn (N)
102	Black Cr., Hwy 29	Perry	Janice (N)
103	Black Cr., Fairly Bridge Landing	Perry	Deep Creek (E)
104	Black Cr., Fairly Bridge Rd.	Perry	Deep Creek (E)
105	Black Cr., Hwy 26	Stone	Wiggins (W)
106	Black Cr., Hwy 57	George	Vestry (N)
107	Red Cr., Hurricane Cr. Rd.	Forrest	Lumberton (W)
108	Red Cr., Stump Texas Rd.	Stone	Wiggins (E)
109	Red Cr., Hwy 26	Stone	Wiggins (E)
110	Red Cr., City Bridge Rd.	Stone	Perkinston (W)
111	Red Cr., Hwy 15	Stone	Ramsey Springs (S)
112	Red Cr., Vestry Rd.	George	Vestry (S)
113	Red Cr., Hwy 57	Jackson	Vestry (W)
114	Pascagoula River, Wade-Vancleave Rd.	Jackson	Wade (E)
115	Escatawpa River, Hwy 612	George	Agricola (W)
116	Escatawpa River, Tanner- Williams Rd.	Jackson	Harleston (W)
117	Escatawpa River, Hwy 614	Jackson	Hurley (W)
118	Atkisson Creek, Old Hwy 24	Greene	McClain (S)
119	Gaines Creek, Arlington Rd	Perry	Beaumont (W)
120	Gaines Creek, Hintonville Rd	Perry	Hintonville (W)
121	Gaines Creek, Cochrane Rd	Perry	Richton (NW)
122	Piney Woods Creek, Brewer/ Otho Sellers Rd.	Perry	Brewer (N)
123	Sandhill Creek, Brewer/ Otho Sellers Rd.	Perry	Brewer (N)
124	Bucatunna Creek, Dyess Bridge Rd.	Wayne	Denham (S)
125	Bucatunna Creek, MS Hwy 510	Wayne	Isney, AL (E)
126	Bucatunna Creek, Old Coyt Rd.	Wayne	Matherville (W)
127	Bucatunna Creek, Clarke Co. 630	Clarke	Melvin, AL (E)
128	Bucatunna Creek, Clarke Co. 511	Clarke	Crandell (W)
129	Bucatunna Creek, Linton Rd.	Clarke	Linton (W)
130	Long Creek, Hwy 18	Clarke	Sykes (E)
131	Bucatunna Creek, Hwy 18	Clarke	Sykes (W)
132	Long Creek, Clarke Co. 514	Clarke	Middleton (E)
133	Bucatunna Creek, Clarke Co. 514	Clarke	Middleton (W)
134	Hurricane Creek, Clarke Co. 430	Clarke	Energy (N)
135	Bucatunna Creek, Vimville-Causeyville Rd.	Lauderdale	Vimville (N)
136	Sowashee Creek, I-20 Exit Ramp (Exit 151)	Lauderdale	Meridian (N)
137	Sowashee Creek, Valley Rd	Lauderdale	Meridian (N)
138	Sowashee Creek, Russell Dr.	Lauderdale	Meridian (W)
139	Sowashee Creek, 23rd St.	Lauderdale	Meridian (W)
140	Sowashee Creek, Marion-Russell Rd.	Lauderdale	Marion (W)
141	Souinlovey Creek, Hwy 513	Jasper	Rose Hill (SW)

Site	Location	County	Nearest Town (Direction)
142	Souinlovey Creek, Hwy 503	Jasper	Hero (N)
143	Souinlovey Creek, Jasper Co. Rd. 31	Jasper	Rose Hill (S)
144	Souinlovey Creek, US Hwy 11	Clarke	Pachuta (S)
145	Tallahala Creek, US Hwy 84	Jones	Laurel (W)
146	Tallahala Creek, US Hwy 11	Jones	Laurel (W)
147	Tallahala Creek, Sandersville-Sharon Rd.	Jones	Sharon (W)
148	Tallahala Creek, Jasper Co. Rd 8	Jasper	Heidelburg (E)
149	Tallahala Creek, Hwy 528	Jasper	Waldrop (E)
150	Nuakfuppa Creek, Hwy 528	Jasper	Acme (W)
151	Tallahoma Creek, Hwy 528	Jasper	Bay Springs (W)
152	Tallahoma Creek, Hwy 537	Jasper	Moss (W)
153	Tallahoma Creek, Shady Grove-Moss Rd	Jones	Moss (N)
154	Tallahoma Creek, Trace Rd.	Jones	Laurel (E)
155	Tallahoma Creek, Hwy 15	Jones	Laurel (E)
156	Tallahoma Creek, Bush Dairy Rd	Jones	Laurel (E)
157	Tallahoma Creek, Flynt Dr	Jones	Laurel (E)
158	Tallahoma Creek, US Hwy 84	Jones	Laurel (E)
159	Tallahoma Creek, Indian Springs Rd	Jones	Pendorff (E)
160	Tallahoma Creek, Burnt Bridge Rd	Jones	Walters (E)
161	Tallahoma Creek, US Hwy 11	Jones	Ellisville (S)

APPENDIX B

ALLELE FREQUENCIES AMONG SIX SAMPLE SITES FOR *GRAPTEMYS FLAVIMACULATA* WITH SIX TESTED MICROSATELLITE LOCI

Locus	Allele	Pascagoula	Lower Chickasawhay	Upper Leaf	Escatawpa	Upper Chickasawhay	Lower Leaf
TerpSH2	**173**	0.074	0.008	0.098	-	-	0.023
	177	0.398	0.425	0.373	0.531	0.595	0.432
	181	0.093	0.100	0.098	0.063	0.048	0.023
	185	0.102	0.042	0.186	0.188	-	0.045
	189	0.259	0.350	0.196	0.219	0.333	0.364
	193	0.028	0.017	0.010	-	0.024	0.068
	197	0.037	0.008	0.029	-	-	0.023
	201	0.009	0.050	0.010	-	-	0.023
	N	54	60	51	32	21	22
	A	8	8	8	4	4	8
	N_e	3.962	3.147	4.306	2.709	2.136	3.054
	A_R	6.793	5.828	6.419	3.968	3.840	7.239
	H_O	0.704	0.600	0.686	0.625	0.429	0.727
	H_E	0.748	0.682	0.768	0.631	0.532	0.673
TerpSH5	**150**	-	**0.008**	-	-	-	-
	154	0.194	0.288	0.452	0.258	0.143	0.447
	158	0.287	0.280	0.183	0.194	0.452	0.184
	162	0.046	0.051	0.058	0.048	0.095	0.079
	166	-	-	0.058	-	-	0.026
	170	0.074	0.059	0.038	0.323	0.071	-
	174	0.037	0.068	0.087	0.016	0.024	0.053
	178	0.287	0.153	0.115	0.048	0.190	0.079
	182	0.037	0.093	0.010	0.048	0.024	0.026
	186	0.028	-	-	-	-	0.079
	190	0.009	-	-	0.065	-	0.026
	N	54	59	52	31	21	19
	A	9	8	8	8	7	9
	N_e	4.677	4.903	3.750	4.555	3.615	3.882
	A_R	7.498	7.063	7.003	7.348	6.712	8.841
	H_O	0.833	0.831	0.750	0.806	0.857	0.895
	H_E	0.786	0.796	0.733	0.780	0.723	0.742
TerpSH7	**109**	**0.028**	-	-	-	-	-
	117	0.037	0.017	-	0.156	0.025	0.024
	121	0.111	0.186	0.115	0.250	0.250	0.190
	125	0.231	0.203	0.240	0.141	0.100	0.262
	129	0.111	0.186	0.221	-	0.050	0.071
	133	0.148	0.136	0.173	0.266	0.275	0.119
	137	0.139	0.136	0.125	0.094	0.050	0.143
	141	0.093	0.051	0.038	0.016	-	0.048
	145	0.037	0.059	0.048	0.016	0.175	0.143
	149	0.009	0.017	0.019	0.047	0.075	-

Locus	Allele	Pascagoula	Lower Chickasawhay	Upper Leaf	Escatawpa	Upper Chickasawhay	Lower Leaf
TerpSH7	**153**	0.056	0.008	0.010	-	-	-
	157	-	-	**0.010**	-	-	-
	161	-	-	-	**0.016**	-	-
	N	54	59	52	32	20	21
	A	11	10	10	9	8	8
	N_e	7.420	6.476	5.885	5.292	5.263	5.959
	A_R	9.549	8.161	7.970	7.604	7.884	7.838
	H_O	0.889	0.915	0.750	0.750	0.950	0.857
	H_E	0.865	0.846	0.830	0.811	0.810	0.832
GmuB08	**230**	0.028	0.017	0.020	0.016	0.024	-
	233	0.259	0.317	0.363	0.129	0.119	0.205
	236	0.296	0.283	0.245	0.177	0.429	0.136
	239	0.259	0.317	0.294	0.565	0.333	0.409
	242	0.139	0.058	0.069	0.081	0.095	0.205
	245	0.019	0.008	0.010	-	-	0.045
	248	-	-	-	**0.032**	-	-
	N	54	60	51	31	21	22
	A	6	6	6	6	5	5
	N_e	4.122	3.514	3.529	2.669	3.139	3.681
	A_R	5.264	4.736	4.894	5.398	4.857	4.970
	H_O	0.704	0.867	0.745	0.613	0.714	0.909
	H_E	0.757	0.715	0.717	0.625	0.681	0.728
GmuD70	**226**	-	-	0.021	-	0.075	-
	230	0.056	0.121	0.146	-	0.125	-
	234	0.037	0.052	0.042	0.094	0.100	0.083
	238	0.139	0.043	0.125	0.063	0.050	0.139
	242	0.102	0.233	0.156	0.063	0.150	0.111
	246	0.065	0.060	0.083	0.109	0.025	0.083
	250	-	**0.009**	-	-	-	-
	274	-	-	-	-	**0.025**	-
	278	-	**0.009**	-	-	-	-
	282	0.065	0.009	0.010	-	-	0.028
	286	0.093	0.121	0.010	0.016	-	-
	290	0.213	0.216	0.063	0.203	0.050	0.222
	294	0.185	0.095	0.167	0.406	0.225	0.139
	298	0.037	0.034	0.104	0.047	0.175	0.167
	302	0.009	-	0.052	-	-	0.028
	306	-	-	**0.021**	-	-	-
	N	54	58	48	32	20	18
	A	11	12	13	8	10	9
	N_e	7.564	6.741	8.597	4.214	7.080	6.968
	A_R	9.738	9.370	10.654	7.412	9.784	9.000
	H_O	0.870	0.810	0.979	0.750	0.750	0.944
	H_E	0.868	0.852	0.884	0.763	0.859	0.856
GmuD88	**128**	0.057	0.044	-	0.125	-	0.083
	132	0.264	0.307	0.347	0.219	0.310	0.250
	136	0.057	0.026	0.041	-	-	0.028

Locus	Allele	Pascagoula	Lower Chickasawhay	Upper Leaf	Escatawpa	Upper Chickasawhay	Lower Leaf
GmuD88	**140**	-	0.009	-	0.047	-	-
	144	0.132	0.096	0.092	0.047	0.119	0.056
	148	0.132	0.219	0.133	0.172	0.214	0.167
	152	0.113	0.114	0.112	0.063	0.143	0.028
	156	0.085	0.044	0.122	0.094	-	0.056
	160	0.057	0.061	0.051	0.063	0.024	0.056
	164	0.075	0.035	0.092	0.125	0.143	0.083
	168	0.009	0.018	-	-	-	-
	172	0.019	0.026	0.010	0.047	0.048	0.194
	N	53	57	49	32	21	18
	A	11	12	9	10	7	10
	N_e	7.120	5.705	5.353	7.585	5.011	6.545
	A_R	9.615	9.634	8.083	9.693	6.840	10.000
	H_O	0.906	0.860	0.857	0.875	0.762	0.944
	H_E	0.860	0.825	0.813	0.868	0.800	0.847

APPENDIX C

SCIENTIFIC COLLECTING PERMIT, 2005

MISSISSIPPI Museum of Natural Science

1 March 2005

ADMINISTRATIVE SCIENTIFIC COLLECTION PERMIT

TO WHOM IT MAY CONCERN:

Permission is granted to:

Will Selman
University of Southern Mississippi
Biological Sciences Department
118 College Drive #5018
Hattiesburg, MS 39406-0001

assisted by Dr. Carl Qualls, Krista Noel, Danna Smith, Thomas Mohrman, and Daniel McGrew is permitted to collect the state endangered, federally threatened yellow-blotched sawback turtle (*Graptemys flavimaculata*) and ringed map turtle (*Graptemys oculifera*) from the Pascagoula and Pearl River drainages (including tributary streams), respectively. Turtles will be live-captured in basking traps, marked, measured, and will have a 1cc blood sample removed for hormone and DNA analysis. All animals will then be promptly released at the point of capture. In the Pascagoula River it is possible that the federally and state endangered Alabama redbelly turtle (*Pseudemys alabamensis*) may be incidentally captured; these should be released following marking, blood sample removal (optional), and photo-documentation (contact Tom Mann ((601) 354-6367, ext. 116) regarding diagnostic features to be photographed). Incidental capture of non-listed turtle species is also authorized.

Collection gear left unattended in the field must be properly identified.

Note: waters south of I-10 are within the jurisdiction of the MS Dept. of Marine Resources (DMR). If collections are to be made within this area, a permit is <u>also</u> necessary from DMR. Contact:

Traci Floyd (228) 374-5022, ext. 5142
Mississippi Dept. of Marine Resources
1141 Bayview Av., Suite 101
Biloxi, MS 39530

With the exceptions noted above, this permit does not authorize the taking of any threatened or endangered species (state or federal, list attached). Because this research is funded by Section 6 research grants from the U.S. Fish and Wildlife Service to the State of Mississippi, Mr. Selman and his assistants are to be regarded as Agents of the State of Mississippi while pursuing this research.

This permit or a copy of this permit must be in possession of the permittee(s) when organisms are taken or possessed under the conditions of the permit.

A complete report of collections must be sent to the Mississippi Museum of Natural Science, 2148 Riverside Dr., Jackson, MS 39202-1353 (Attn: Scientific Collection Permit Review Committee) within 15 days of permit expiration. Collection reports should list taxa collected, number of individuals of each, exact collection locality and date of collection. Locality information must include the county of collection, and it is preferred that precise locality

Preserving Natural Mississippi

2148 RIVERSIDE DRIVE • JACKSON, MS 39202-1353 • **PHONE** 601 354-7303 **FAX** 601 354-7227 • www.mdwfp.state.ms.us/museum

DEPARTMENT OF WILDLIFE, FISHERIES, AND PARKS

information be provided in latitude/longitude (GPS) or in the township, range, and section (TRS) system. If the TRS system is used, precise location within a section should be indicated (e.g.: NW4 of SE4 of Sec 11), if possible. If GPS or TRS information is not provided, include instead a clear and precise description of the location of the collection site relative to the nearest named or numbered road, town, intersection, and/or other feature(s) likely to be mapped on a USGS quad map. For aquatic species, provide the name of the stream in which collections were made.

All individuals of listed species will be released at the point of collection. Individuals of non-listed species may be released or may be retained for vouchers if viewed as significant locality records by Dr. Bob Jones of the Mississippi Museum of Natural Science ((601) 354-6367, ext. 113). Such voucher specimens should be deposited at the Mississippi Museum of Natural Science or in another curated collection where they remain available for general scientific study.

A copy of publications, survey reports, and other printed materials produced as a result of this collection should be sent to the Mississippi Museum of Natural Science (Attn: Scientific Collection Permit Review Committee).

This permit expires one year from date of issuance, on 28 February 2006.

Libby Hartfield

Libby Hartfield, Director
Mississippi Museum of Natural Science

LH:tm, conservation biology section

cc: Gary Berry, Manager, Region 2 and Region 2
Museum

Enclosure

APPENDIX D

SCIENTIFIC COLLECTING PERMIT, 2005

MISSISSIPPI Museum of Natural Science

21 July 2005

MODIFIED ADMINISTRATIVE SCIENTIFIC COLLECTION PERMIT

TO WHOM IT MAY CONCERN:

Permission is granted to:

Will Selman
University of Southern Mississippi
Biological Sciences Department
118 College Drive #5018
Hattiesburg, MS 39406-0001

assisted by Dr. Carl Qualls, Krista Noel, Danna Smith, Thomas Mohrman, Daniel McGrew, Sam Wilson, and Ted Wilson, is permitted to collect the state endangered, federally threatened yellow-blotched sawback turtle (*Graptemys flavimaculata*) and ringed map turtle (*Graptemys oculifera*) from the Pascagoula and Pearl River drainages (including tributary streams), respectively. Turtles will be live-captured in basking traps, marked, measured, and will have a 1cc blood sample removed for hormone and DNA analysis. All animals will then be promptly released at the point of capture. In the Pascagoula River it is possible that the federally and state endangered Alabama redbelly turtle (*Pseudemys alabamensis*) may be incidentally captured; these should be released following marking, blood sample removal (optional), and photo-documentation (contact Tom Mann ((601) 354-6367, ext. 116) regarding diagnostic features to be photographed). Incidental capture of non-listed turtle species is also authorized.

Collection gear left unattended in the field must be properly identified.

> **Note: waters south of I-10 are within the jurisdiction of the MS Dept. of Marine Resources (DMR). If collections are to be made within this area, a permit is <u>also</u> necessary from DMR. Contact:**
>
> **Traci Floyd (228) 374-5022, ext. 5142**
> **Mississippi Dept. of Marine Resources**
> **1141 Bayview Av., Suite 101**
> **Biloxi, MS 39530**

With the exceptions noted above, this permit does not authorize the taking of any threatened or endangered species (state or federal, list attached). Because this research is funded by Section 6 research grants from the U.S. Fish and Wildlife Service to the State of Mississippi, Mr. Selman and his assistants are to be regarded as Agents of the State of Mississippi while pursuing this research.

This permit or a copy of this permit must be in possession of the permittee(s) when organisms are taken or possessed under the conditions of the permit.

A complete report of collections must be sent to the Mississippi Museum of Natural Science, 2148 Riverside Dr., Jackson, MS 39202-1353 (Attn: Scientific Collection Permit Review Committee) within 15 days of permit expiration. Collection reports should list taxa collected, number of individuals of each, exact collection locality and date of collection. Locality information must include the county of collection, and it is preferred that precise locality

Preserving Natural Mississippi

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DEPARTMENT OF WILDLIFE, FISHERIES, AND PARKS

information be provided in latitude/longitude (GPS) or in the township, range, and section (TRS) system. If the TRS system is used, precise location within a section should be indicated (e.g.: NW4 of SE4 of Sec 11), if possible. If GPS or TRS information is not provided, include instead a clear and precise description of the location of the collection site relative to the nearest named or numbered road, town, intersection, and/or other feature(s) likely to be mapped on a USGS quad map. For aquatic species, provide the name of the stream in which collections were made.

All individuals of listed species will be released at the point of collection. Individuals of non-listed species may be released or may be retained for vouchers if viewed as significant locality records by Dr. Bob Jones of the Mississippi Museum of Natural Science ((601) 354-6367, ext. 113). Such voucher specimens should be deposited at the Mississippi Museum of Natural Science or in another curated collection where they remain available for general scientific study.

A copy of publications, survey reports, and other printed materials produced as a result of this collection should be sent to the Mississippi Museum of Natural Science (Attn: Scientific Collection Permit Review Committee).

This permit expires one year from date of original issuance, on 28 February 2006.

Charles Knight for Libby Hartfield

Libby Hartfield, Director
Mississippi Museum of Natural Science

LH:tm, conservation biology section

cc: Gary Berry, Manager, Region 2 and Region 2 Museum

Enclosure

APPENDIX E

SCIENTIFIC COLLECTING PERMIT, 2006

MISSISSIPPI Museum of Natural Science

23 March 2006

ADMINISTRATIVE SCIENTIFIC COLLECTION PERMIT

TO WHOM IT MAY CONCERN:

Permission is granted to:

Will Selman
University of Southern Mississippi
Biological Sciences Department
118 College Drive #5018
Hattiesburg, MS 39406-0001,

assisted by Dr. Carl Qualls, Krista Noel, Brian Kreiser, Danna Smith, Thomas Mohrman, Daniel McGrew, Mike Sisson and Josh Ennen, to collect the state endangered, federally threatened yellow-blotched map turtle, primarily with basking traps, to obtain blood samples. In addition, this and other turtle species may also be captured with hoop nets and dip nets, but hoop nets must be secured and monitored so as to preclude drowning of these or incidentally captured species. All animals will be promptly released at the point of capture after removal of blood samples. Collecting will be done in the Pascagoula, Pearl, Leaf, Chickasawhay and Escatawpa Rivers and their tributaries. In the Pearl River it is possible that the state endangered, federally threatened ringed map turtle (*Graptemys oculifera*) may be incidentally captured; these should be released immediately following examination for previous marking by Dr. Bob Jones. In the Pascagoula River it is possible that the federally and state endangered Alabama redbelly turtle (*Pseudemys alabamensis*) may be incidentally captured; these should be released following examination for previous marking by other researchers, blood sample removal (optional), and photo-documentation (contact Tom Mann ((601) 354-6367, ext. 116) regarding diagnostic features to be photographed and for marginal notching system used in previous research on this species).

Ownership of collection gear left unattended in the field must be clearly marked.

Note: waters south of I-10 are within the jurisdiction of the MS Dept. of Marine Resources (DMR). If collections are to be made within this area, a permit is <u>also</u> necessary from DMR. Contact:

Traci Floyd (228) 374-5022, ext. 5142
Mississippi Dept. of Marine Resources
1141 Bayview Av., Suite 101
Biloxi, MS 39530

With the exceptions noted above, this permit does not authorize the taking of any threatened or endangered species (state or federal, list attached). Because this research is funded by Section 6 research grants from the U.S. Fish and Wildlife Service to the State of Mississippi, Mr. Selman and his assistants are to be regarded as Agents of the State of Mississippi while pursuing this research.

This permit or a copy of this permit must be in possession of the permittee(s) when organisms are taken or possessed under the conditions of the permit.

Preserving Natural Mississippi

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DEPARTMENT OF WILDLIFE, FISHERIES, AND PARKS

A complete report of collections must be sent to the Mississippi Museum of Natural Science, 2148 Riverside Dr., Jackson, MS 39202-1353 (Attn: Scientific Collection Permit Review Committee) within 15 days of permit expiration. Collection reports should list taxa collected, number of individuals of each, exact collection locality and date of collection. Locality information must include the county of collection, and it is preferred that precise locality information be provided in latitude/longitude (GPS) or in the township, range, and section (TRS) system. If the TRS system is used, precise location within a section should be indicated (e.g.: NW4 of SE4 of Sec 11), if possible. If GPS or TRS information is not provided, include instead a clear and precise description of the location of the collection site relative to the nearest named or numbered road, town, intersection, and/or other feature(s) likely to be mapped on a USGS quad map. For aquatic species, provide the name of the stream in which collections were made.

All individuals of listed species will be released at the point of collection. Individuals of non-listed species may be released or may be retained for vouchers if viewed as significant locality records by Dr. Bob Jones of the Mississippi Museum of Natural Science ((601) 354-6367, ext. 113). Such voucher specimens should be deposited at the Mississippi Museum of Natural Science or in another curated collection where they remain available for general scientific study.

A copy of publications, survey reports, and other printed materials produced as a result of this collection should be sent to the Mississippi Museum of Natural Science (Attn: Scientific Collection Permit Review Committee).

This permit expires one year from date of original issuance, on 22 March 2007.

Libby Hartfield

Libby Hartfield, Director
Mississippi Museum of Natural Science

LH:tm, conservation biology section

cc: Randall Miller, Assistant Chief, Field Operations, MDWFP
Museum

Enclosure

APPENDIX F

SCIENTIFIC COLLECTING PERMIT, 2007

MISSISSIPPI Museum of Natural Science

26 February 2007

ADMINISTRATIVE SCIENTIFIC COLLECTION PERMIT

TO WHOM IT MAY CONCERN:

Permission is granted to:

Will Selman
University of Southern Mississippi
Biological Sciences Department
118 College Drive #5018
Hattiesburg, MS 39406-0001,

assisted by Dr. Carl Qualls, Brian Kreiser, Danna Baxley, Thomas Mohrman, Brian Horne, Greg George, Mike Sisson, Josh Ennen, and supervised field technicians, to collect the state endangered, federally threatened yellow-blotched map turtle (*Graptemys flavimaculata*), primarily with basking traps, to obtain blood samples (including corticosterone stress challenge sample series), and to affix to some animals external transmitters to monitor location and body temperature. The state endangered, federally threatened ringed map turtle (*Graptemys oculifera*) may also be captured for blood samples for DNA analysis. All animals will be promptly released at the point of capture after removal of blood samples, weighing, permanent scute marking, and paint-marking (during October population surveys). In addition, these and other turtle species may also be captured with hoop nets and dip nets, but hoop nets must be secured and monitored so as to preclude drowning of these or incidentally captured species. Collecting will be done in the Pascagoula, Pearl, Leaf, Chickasawhay and Escatawpa Rivers and their tributaries. In the Pascagoula River it is possible that the federally and state endangered Alabama redbelly turtle (*Pseudemys alabamensis*) may be incidentally captured; these should be released following examination for previous marking by other researchers, blood sample removal (optional), and photo-documentation (contact Tom Mann ((601) 354-6367, ext. 116) regarding diagnostic features to be photographed and for marginal notching system used in previous research on this species).

Ownership of collection gear left unattended in the field must be clearly marked.

Note: waters south of I-10 are within the jurisdiction of the MS Dept. of Marine Resources (DMR). If collections are to be made within this area, a permit is also necessary from DMR. Contact:

Traci Floyd (228) 374-5022, ext. 5142
Mississippi Dept. of Marine Resources
1141 Bayview Av., Suite 101
Biloxi, MS 39530

With the exceptions noted above, this permit does not authorize the taking of any threatened or endangered species (state or federal, list attached). Because this research is funded by Section 6 research grants from the U.S. Fish and Wildlife Service to the State of Mississippi, Mr. Selman and his assistants are to be regarded as Agents of the State of Mississippi while pursuing this research.

Preserving Natural Mississippi

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DEPARTMENT OF WILDLIFE, FISHERIES, AND PARKS

This permit or a copy of this permit must be in possession of the permittee(s) when organisms are taken or possessed under the conditions of the permit.

A complete report of collections must be sent to the Mississippi Museum of Natural Science, 2148 Riverside Dr., Jackson, MS 39202-1353 (Attn: Scientific Collection Permit Review Committee) within 15 days of permit expiration. Collection reports should list taxa collected, number of individuals of each, exact collection locality and date of collection. Locality information must include the county of collection, and it is preferred that precise locality information be provided in latitude/longitude (GPS) or in the township, range, and section (TRS) system. If the TRS system is used, precise location within a section should be indicated (e.g.: NW4 of SE4 of Sec 11), if possible. If GPS or TRS information is not provided, include instead a clear and precise description of the location of the collection site relative to the nearest named or numbered road, town, intersection, and/or other feature(s) likely to be mapped on a USGS quad map. For aquatic species, provide the name of the stream in which collections were made.

All individuals of listed species will be released at the point of collection. Individuals of non-listed species may be released or may be retained for vouchers if viewed as significant locality records by Dr. Bob Jones of the Mississippi Museum of Natural Science ((601) 354-6367, ext. 113). Such voucher specimens should be deposited at the Mississippi Museum of Natural Science or in another curated collection where they remain available for general scientific study.

A copy of publications, survey reports, and other printed materials produced as a result of this collection should be sent to the Mississippi Museum of Natural Science (Attn: Scientific Collection Permit Review Committee).

This permit expires one year from date of original issuance, on 25 February 2008.

Charles Knight for Libby Hartfield

Libby Hartfield, Director
Mississippi Museum of Natural Science

LH:tm, conservation biology section

cc: Randall Miller, Assistant Chief, Field Operations, MDWFP
Museum

Enclosure

APPENDIX G

SCIENTIFIC COLLECTING PERMIT, 2008

MISSISSIPPI
DEPARTMENT OF WILDLIFE, FISHERIES, AND PARKS

Sam Polles, Ph.D.
Executive Director

26 March 2008

ADMINISTRATIVE SCIENTIFIC COLLECTION PERMIT NUMBER 0326091

TO WHOM IT MAY CONCERN:

Permission is granted to:

Will Selman
University of Southern Mississippi
Biological Sciences Department
118 College Drive #5018
Hattiesburg, MS 39406-0001,

assisted by Dr. Carl Qualls, Brian Kreiser, Josh Ennen, and supervised field technicians, to collect the state endangered, federally threatened yellow-blotched map turtle (*Graptemys flavimaculata*), to obtain blood samples (including corticosterone stress challenge sample series), and to affix to some animals external transmitters to monitor location and body temperature. The state endangered, federally threatened ringed map turtle (*Graptemys oculifera*) may also be captured for blood samples for DNA analysis.
Additionally, data and tissue samples may be taken from Pascagoula map turtles, river cooters, red-eared sliders, razorback musk turtles, musk turtles, softshell turtles, common snapping turtles, and alligator snapping turtles Also, the state and federally endangered Alabama redbellied turtle may handled as described in (6) below.

This permit is valid from 30 march 2009 to 29 March 2010

SPECIFIC CONDITIONS AND RESTRICTIONS

1.) All animals will be promptly released at the point of capture after removal of blood samples, weighing, permanent scute marking, and paint-marking (during October population surveys).

2.) Turtles will be captured using basking traps, dip nets, and hoop nets.

3.) Hoop nets must be monitored secured and regularly monitored to prevent drowning of captured turtles.

Mississippi Museum of Natural Science ● 2148 Riverside Drive ● Jackson, Mississippi 39202-1353 ● (601) 354-7303

4.) All collecting gear left unattended must be labeled with the collector's name, institution, and permit number.

5.) Collecting will be done in the Pascagoula, Pearl, Leaf, Chickasawhay and Escatawpa Rivers and their tributaries.

6.) In the Pascagoula River it is possible that the federally and state endangered Alabama redbelly turtle (*Pseudemys alabamensis*) may be incidentally captured; these should be released following examination for previous marking by other researchers, blood sample removal (optional), and photo-documentation (contact Tom Mann ((601) 354-6367, ext. 116) regarding diagnostic features to be photographed and for marginal notching system used in previous research on this species).

GENERAL CONDITIONS AND RESTRICTIONS:

1) Specimens retained after collection must be placed in a public museum or collection where they will be available for examination by the scientific community. The Mississippi Museum of Natural Science (MMNS), 2148 Riverside Drive, Jackson, MS 39202-1353, ph: (601) 354-7303, is the principal repository of terrestrial and freshwater vertebrates, freshwater mollusks, and crayfish collected in Mississippi, and welcomes additional specimens. **Unless alternative arrangements are made with the MMNS Collections manager (Scott Peyton, 601-354-7303) or curatorial staff at the MMNS, all collections of federally listed and state listed species will be deposited at the Mississippi Museum of Natural Science.**

2) **This permit does not authorize the taking of any federally threatened or endangered species or any state endangered species (list attached), unless otherwise specified in this permit.**

3) All wildlife, including fish and invertebrates, collected under the permit are considered to be a natural resource of the State of Mississippi. Collected specimens should be handled humanely, and live, uninjured specimens not needed for permanent collections should be returned to appropriate habitat at the capture locality when no longer required. Specimens that die incidental to collection activities or which are intentionally preserved must be maintained in a scientifically acceptable fashion in a study/research collection where they will be available for examination by the general scientific community, or should be offered to a museum. The intent of the scientific collecting permit is to encourage meaningful study and to discourage the loss of specimens and information.

4) The issuance of a permit does not authorize trespass by the permittee. Permit is also void if permittee has not obtained other necessary permissions/permits for collection activities on public lands.

5) Collection of migratory birds, their nests, or eggs, collection of federally listed endangered species, and collection of federally listed threatened species (when the

collector is not an agent of the State of Mississippi) requires a federal permit in addition to a state permit.

6) Copies of publications, survey reports, and other printed materials produced as a result of this collection should be sent to the Mississippi Museum of Natural Science (Attn: Scientific Collection Permit Review Committee.) 2148 Riverside Dr., Jackson, MS 39202.

REQUIRED COLLECTING PERMIT REPORTS

1) **A collecting permit report using format described below must be filed within 15 days of the expiration of the permit. A new permit will not be issued until the report has been received. Collection reports should list taxa collected, number of individuals of each, exact collection locality and date of collection. Locality information must include the county of collection, and it is preferred that precise locality information be provided in latitude/longitude (GPS) or in the township, range, and section (TRS) system. If the TRS system is used, precise location within a section should be indicated (e.g.: NW4 of SE4 of Sec 11), if possible. If GPS or TRS information is not provided, include instead a clear and precise description of the location of the collection site relative to the nearest named or numbered road, town, intersection, and/or other feature(s) likely to be mapped on a USGS quad map. For aquatic species, provide the name of the stream in which collections were made.**

Instructions for completing Scientific Collections Report

Below is a list of information that should be included in scientific collecting reports, if it applies to the activities covered by the collecting permit. Because of the broad spectrum of activities covered by collecting permits, individual reports may require an altered format or other information not described below. If possible, reports should be submitted electronically in a spreadsheet format (preferably in Excel or Access). A blank spreadsheet with the requested fields can be provided to you by Email. Please include the following fields in the spreadsheet, if they apply to the work conducted under the permit. If you cannot provide an electronic version of the collections report, a blank hard copy of a collections report form can be provided to you. If you have any questions, please contact Scott Peyton at 601-354-7303 or collections.manager@mmns.state.ms.us.

A. SPECIES - species name (scientific name), or lowest taxonomic description possible, for each collected taxon.
B. SACRIFICED - If specimens were killed for vouchers or other scientific purposes, indicate the number taken.
C. NUMBER – total number of each species collected or handled. Include both the number taken and the number released in this total.
D. DATE – specific date of each collection.
E. COUNTY – county where each collection occurred.
F. COORDINATES (X) - latitude/longitude, UTM coordinates
G. COORDINATES (Y) - latitude/longitude, UTM coordinates

H. UTM ZONE – UTM coordinates only
I. TRS - Township, Range and Section (optional, but please include if possible)
J. LOCALITY - brief description of locality, e.g. Chickasawhay River 100m upstream from HWY 84 bridge.
K. COLLECTOR(S) – person or persons who made the collection.
L. TISSUE - Indicate the number of specimens from which tissue samples were taken for genetic analysis or other purposes. If no tissue samples were taken, this column can be omitted.
M. DISPOSITION - For sacrificed specimens or tissue samples, list institution(s) where specimens/samples were deposited. For specimens released, indicate where the specimens were released.
N. TEMP EXP or TEMP PROP - If specimens are held in captivity temporarily for experimental purposes or for propagation and later released, a field should be included to capture this information.
O. TAGGED - If specimens are marked or tagged and released, a field should be included to capture this information.

2) **Those collecting federally listed species specified in this permit must submit an additional report to the state, due the first week of October, detailing collections of listed species made between 1 October of the previous year and 30 September of the current year.**

Libby Hartfield

Libby Hartfield, Director
Mississippi Museum of Natural Science
Mississippi Department of Wildlife, Fisheries, & Parks

LH:ly, conservation biology section
cc: Randall Miller, Assistant Chief, Field Operations, MDWFP
Museum

Enclosure

Enclosure

APPENDIX H

SALTWATER SCIENTIFIC COLLECTING PERMIT, 2006

MISSISSIPPI
DEPARTMENT OF MARINE RESOURCES

Saltwater Scientific Collection Permit

Date: July 19, 2006

ISSUED TO: WILL SELMAN, DEPARTMENT OF BIOLOGICAL SCIENCES GRADUATE STUDENT, THE UNIVERSITY OF SOUTHERN MISSISSIPPI, 118 COLLEGE DRIVE #5018, HATTIESBURG, MS 39406-0001.

PERSONNEL: WILL SELAMAN, CARL QUALLS, DANNA BAXLEY, THOMAS MOHRMAN, JOSHUA ENNEN, BRIAN KREISER, AND TOMMY HUFF.

DESCRIPTION: AS PART OF STUDY "CONSERVATION GENETICS OF THE YELLOW-BLOTCHED SAWBACK TURTLE", A TARGET OF 40 BLOOD SAMPLES EACH FROM YELLOW-BLOTCHED SAWBACK (GRAPTEMYS FLAVIMACULATA) AND RIVER COOTER (PSEUDEMYS CONCINNA) TURTLES WILL BE COLLECTED.

- GEAR SHALL BE LIMITED TO BASKING TRAPS, HOOP NETS, AND DIP NETS.
- PRIOR TO SAMPLING THE MARINE PATROL OFFICE MUST BE CONTACTED *DAILY* AT 228-432-7708.
- COLLECTION WILL BE CONDUCTED SOUTH OF I-10 ON THE ESCATAWPA RIVER.
- NONE OF THE SPECIMENS COLLECTED UNDER THIS PERMIT ARE TO BE SOLD, BARTERED, TRADED OR CONSUMED.
- ALL SPECIMENS WHICH ARE NOT RETAINED AS VOUCHER SPECIMENS MUST BE RETURNED TO THE WATER AS SOON AS POSSIBLE.
- VOUCHER SPECIMENS WILL BE DEPOSITED IN THE COLLECTIONS OF THE MISSISSIPPI MUSEUM OF NATURAL SCIENCE.
- NO PROTECTED OR ENDANGERED SPECIES ARE TO BE RETAINED WITH THIS PERMIT.
- A WRITTEN REPORT SUMMARIZING THIS PROJECT IS DUE TO THE DMR OFFICE WITHIN 60 DAYS OF ITS EXPIRATION.

THIS PERMIT MAY BE CANCELLED AT ANY TIME IF IN THE JUDGMENT OF THE EXECUTIVE DIRECTOR THAT THIS PERMIT IS BEING USED FOR PURPOSES OTHER THAN THAT WHICH IT WAS INTENDED. THIS PERMIT OR A COPY OF THIS PERMIT MUST BE IN POSSESSION WHEN TAKING OR POSSESSING ORGANISMS UNDER THE CONDITIONS OF THE PERMIT.

APPROVED BY:

WILLIAM S. "CORKY" PERRET, MARINE FISHERIES DIRECTOR

CC: MARINE PATROL

THIS PERMIT EXPIRES DECEMBER 31, 2006

1141 Bayview Avenue • Biloxi, MS 39530-1613 • Tel: (228) 374-5000 • www.dmr.state.ms.us
An Equal Opportunity Employer

APPENDIX I

SALTWATER SCIENTIFIC COLLECTING PERMIT, 2007

MISSISSIPPI
DEPARTMENT OF MARINE RESOURCES

Saltwater Scientific Collection Permit

Date: March 1, 2007

ISSUED TO: WILL SELMAN, GRADUATE STUDENT, UNIVERSITY OF SOUTHERN MISSISSIPPI, 118 COLLEGE DRIVE #5018, HATTIESBURG, MS 39406-0001. PHONE: (601) 466-6114

PERSONNEL: WILL SELMAN, CARL QUALLS, DANNA BAXLEY, THOMAS MOHRMAN, JOSHUA ENNEN, BRIAN KREISER, AND SUPERVISED FIELD TECHNICIANS.

DESCRIPTION: GENETICS STUDY OF THE YELLOW-BLOTCHED SAWBACK TURTLE (*GRAPTEMYS FLAVIMACULATA*) AND THE RIVER COOTER (*PSEUDEMYS CONCINNA*) MAINLY IN TRIBUTARIES AND ADJACENT WATERS OF THE LEAF RIVER, THE LOWER PASCAGOULA RIVER AND THE ESCATAWPA RIVER.

- SAMPLING SHALL BE CONCUCTED USING BASKING TRAPS (MARKED "USM"), HOOP NETS, AND DIP NETS.
- TURTLES SHALL BE HELD TEMPORARILY FOR MEASUREMENTS AND PROCESSING.
- COLLECTION VESSELS SHALL BE IDENTIFIED AS: #MI-0430-BK, SERIAL NUMBER #JBC 59179 C505.
- PRIOR TO SAMPLING ACTIVITES, MARINE PATROL SHALL BE GIVEN ADVANCE NOTICE AT (228) 523-4134.
- NONE OF THE SPECIMENS COLLECTED UNDER THIS PERMIT ARE TO BE SOLD, BARTERED, TRADED OR CONSUMED.
- ALL SPECIMENS SHALL BE RETURNED TO THE WATER AS SOON AS POSSIBLE. IN THE EVENT VOUCHER SPECIMENS ARE REQUESTED OR IN THE EVENT OF MORTALITIES, SPECIMENS WILL BE DEPOSITED AT THE MS MUSEUM OF NATURAL SCIENCE.
- NO PROTECTED OR ENDANGERED SPECIES SHALL BE RETAINED WITH THIS PERMIT.
- A COMPLETE WRITTEN REPORT SUMMARIZING COLLECTION ACTIVITY MUST BE SUBMITTED TO THE MARINE FISHERIES OFFICE WITHIN 60 DAYS OF THE EXPIRATION.

THIS PERMIT MAY BE CANCELLED AT ANY TIME IF IN THE JUDGMENT OF THE EXECUTIVE DIRECTOR THAT THIS PERMIT IS BEING USED FOR PURPOSES OTHER THAN THAT WHICH IT WAS INTENDED. THIS PERMIT OR A COPY OF THIS PERMIT MUST BE IN POSSESSION WHEN TAKING OR POSSESSING ORGANISMS UNDER THE CONDITIONS OF THE PERMIT.

APPROVED BY:

Joseph D. Jewell for

DALE A. DIAZ, MARINE FISHERIES DIRECTOR

CC: MARINE PATROL

THIS PERMIT EXPIRES DECEMBER 31, 2007

1141 Bayview Avenue, Suite 101 • Biloxi, MS 39530 • (228) 374-5000

APPENDIX J

INSTITUTE FOR ANIMAL CARE AND USE COMMITTEE APPROVAL

The University of Southern Mississippi
Institutional Animal Care and Use Committee

118 College Drive #5147
Hattiesburg, MS 39406-0001
Tel: 601.266.6820
Fax: 601.266.5509
www.usm.edu/spa/policies/animals

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
NOTICE OF COMMITTEE ACTION

The proposal noted below was reviewed and approved by The University of Southern Mississippi Institutional Animal Care and Use Committee (IACUC) in accordance with regulations by the United States Department of Agriculture and the Public Health Service Office of Laboratory Animal Welfare. The project expiration date is noted below. If for some reason the project is not completed by the end of the three year approval period, your protocol must be reactivated (a new protocol must be submitted and approved) before further work involving the use of animals can be done.

Any significant changes (see attached) should be brought to the attention of the committee at the earliest possible time. If you should have any questions, please contact me.

PROTOCOL NUMBER: **07032201**
PROJECT TITLE: **Assessing the Impacts of Hurricane Katrina on Populations of the Yellow-Blotched Sawback Turtle**
PROPOSED PROJECT DATES: **01/01/07 to 12/31/09**
PROJECT TYPE: **New Project**
PRINCIPAL INVESTIGATOR(S): **Carl Qualls, Ph.D.**
COLLEGE/DIVISION: **College of Science & Technology**
DEPARTMENT: **Biological Sciences**
FUNDING AGENCY/SPONSOR: **U. S. Fish and Wildlife Service (USFWS)**
IACUC COMMITTEE ACTION: **Full Committee Review**
PROTOCOL EXPIRATION DATE: **09/30/09**

Robert C. Bateman, Jr., Ph.D.
IACUC Chair

3-22-07
Date

APPENDIX K

INSTITUTE FOR ANIMAL CARE AND USE COMMITTEE APPROVAL

The University of Southern Mississippi

Institutional Animal Care and Use Committee

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INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
NOTICE OF COMMITTEE ACTION

The proposal noted below was reviewed and approved by The University of Southern Mississippi Institutional Animal Care and Use Committee (IACUC) in accordance with regulations by the United States Department of Agriculture and the Public Health Service Office of Laboratory Animal Welfare. The project expiration date is noted below. If for some reason the project is not completed by the end of the three year approval period, your protocol must be reactivated (a new protocol must be submitted and approved) before further work involving the use of animals can be done.

Any significant changes (see attached) should be brought to the attention of the committee at the earliest possible time. If you should have any questions, please contact me.

PROTOCOL NUMBER: **09093001**
PROJECT TITLE: **Impacts of Hurricane Katrina**
PROPOSED PROJECT DATES: **08/01/2009 – 07/31/2010**
PROJECT TYPE: **Renewal**
PRINCIPAL INVESTIGATOR(S): **Carl Qualls, Ph.D.**
COLLEGE/DIVISION: **College of Science & Technology**
DEPARTMENT: **Biological Sciences**
FUNDING AGENCY/SPONSOR: **U.S. Fish and Wildlife Service (USFWS)**
IACUC COMMITTEE ACTION: **Designated Review**
PROTOCOL EXPIRATION DATE: **09/30/2012**

Robert C Bateman — 9-30-09

Robert C. Bateman, Jr., Ph.D.
IACUC Chair

Date

LITERATURE CITED

Apanius, V. 1998. Stress and immune defense. Advances in the Study of Animal Behavior. 27:133-153.

Auth, D.L. 1975. Behavioral ecology of basking in the yellow-bellied turtle, *Chrysemys scripta scripta* (Schoepff). Bulletin of the Florida State Museum, Biological Sciences 20(1): 1-45.

Ball, G. F. and J.C. Wingfield. 1987. Changes in plasma levels of sex steroids in relation to multiple broodedness and nest site density in male starlings. Physiological Zoology (60):191-199.

Beutler, E., T. Gelbart, and W. Kuhl. 1990. Interference of heparin with the polymerase chain reaction. BioTechniques 9:166.

Bicker, A.R. 1969. Geologic map of Mississippi: U.S. Geological Survey, scale 1:500000.

Boutin-Ganache, I., M. Raposo, M. Raymond, and M. Deschepper. 2001. M-13 tailed primers improved the readability and usability of microsatellite analyses performed with two different allele-sizes methods. Biotechniques 31:24-28.

Bowen, K.D. and F.J. Janzen. 2008. Human recreation and the nesting ecology of a freshwater turtle (*Chrysemys picta*). Chelonian Conservation and Biology 7(1):95-100.

Boyer, D.R. 1965. Ecology of the basking habit in turtles. Ecology 46:99-118.

Boyle, S.A. and Samson, F.B. 1985. Effects of nonconsumptive recreation on wildlife: A review. Wildlife Society Bulletin 13:110–116.

Buck, E.H. 2005. Hurricanes Katrina and Rita: Fishing and aquaculture industries-damage and recovery. CRS report for Congress. 6 pp.

Buhlmann, K. A. and J. W. Gibbons. 1997. Imperiled aquatic reptiles of the southeastern United States: Historical review and current conservation status. *In* Aquatic fauna in peril: The southeastern perspective. G. W. Benz and D. E. Collins (Eds.). Special Publication 1, Southeast Aquatic Research Institute, Lenz Design and Communication, Decatur, GA. pp. 201-232.

Bulté, G. and G. Blouin-Demers. 2009. Implications of extreme sexual size dimorphism for thermoregulation in a freshwater turtle. Oecologia. Published Online. DOI 10.1007/s00442-009-1469-8.

Bulté, G., M.A. Carrière, and G. Blouin-Demers. 2009. Impact of recreational power boating on two populations of northern map turtles (*Graptemys geographica*). Aquatic Conservation: Marine and Freshwater Ecosystems. Published Online. DOI: 10.1002/aqc.1063.

Burger, J. 2001. The behavioral response of basking Northern Water (*Nerodia sipedon*) and Eastern Garter (*Thamnophis sirtalis*) Snakes to pedestrians in a New Jersey park. Urban ecosystems 5:119-129.

Burger, J. and S.D. Garber. 1995. Risk assessment, life history strategies, and turtles: Could declines be prevented or predicted? Journal of Toxicology and Environmental Health 46:483-500.

Bury, R.B. 1972. Habits and home range of the Pacific pond turtle, *Clemmys marmorata*, in a stream community. Ph.D. Dissertation. University of California, Berkeley, California, USA.

Bury, R.B. 2006. Natural history, field ecology, conservation biology and wildlife management: Time to connect the dots. Herpetological Conservation and Biology 1:56-61.

Cagle, F.R. 1950. The life history of the slider turtle, *Pseudemys scripta troostii* (Holbrook). Ecological Monographs 20: 31-54.

Cagle, F.R. 1954. Two new species of the genus *Graptemys*. Tulane Studies in Zoology. 1:167-186.

Chen, T.H., H.C. Lin, and H.C. Chang. 2000. Current status and utilization of chelonians in Taiwan. *In*: Asian Turtle Trade: Proceedings of a workshop on conservation and trade of freshwater turtles and tortoises in Asia. Vol. 2. P.P. van Dijk, B.L. Stuart, and A.G.J. Rhodin (Eds.). Chelonian Research Foundation, Phnom Penh, Cambodia. p. 45-51.

Cliburn, J.W. 1971. The ranges of four species of *Graptemys* in Mississippi. Journal of Mississippi Academy of Sciences 16:16-19.

Coleman, J.L. and R.L. Gutberlet, Jr. 2008. Seasonal variation in basking in two syntopic species of map turtles (Emydidae: *Graptemys*). Chelonian Conservation and Biology 7(2): 276-281.

Congdon, J.D., A.E. Dunham, and R.C. van Loben Sels. 1993. Delayed sexual maturity and demographics of Blanding's turtles (*Emydoidea blandingii*): Implications for conservation and management of long-lived organisms. Conservation Biology 7: 826-833.

Congdon, J.D., A.E. Dunham, and R.C. van Loben Sels. 1994. Demographics of snapping turtles (*Chelydra serpentina*): Implications for conservation and management of long-lived organisms. American Zoologists 34: 397-408.

Cornuet J-M and G. Luikart. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* 144: 2001-2014.

Czech, B., P. R. Krausman, and P. K. Devers. 2000. Economic associations among causes of species endangerment in the United States. BioScience 50(7):593-601.

Dickerson, D.D. and K.J. Reine. 1996. Habitat Assessment and Relative Abundance Estimates for the Ringed Sawback Turtle (*Graptemys oculifera*) in Dredging Sites of the West Pearl River Navigation Project (Louisiana). U.S. Army Engineer District, Vicksburg, MS, 52 pp.

Di Rienzo A, A.C. Peterson, J.C. Garze, A.M. Valdes, M. Slatkin, and N.B. Freimer. 1994. Mutational processes of simple sequence repeat loci in human populations. Proceedings of the National Academy of Sciences 91:3166–3170.

Dynesius, M. and C. Nilsson. 1994. Fragmentation and flow regulation of river systems in the northern third of the world. Science 266:753-761.

Ecksdine, V. 1985. Meteorological influence on fall basking of the map turtle, *Graptemys geographica*, in a north temperate stream. Bulletin of the Chicago Herpetological Society 20(3): 82-85.

Ennen, J.R., W. Selman, and B.R. Kreiser. 2007. *Graptemys gibbonsi*, Diet. Natural History Note. Herpetological Review 38(2):200.

Ernst, C.H. and J. Lovich. 2009. Turtles of the United States and Canada. 2nd ed. Smithsonian Institute Press, Washington D.C.

Excoffier, L., P.E. Smouse and J.M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics 131: 479-491.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47-50.

Fitch, H.S. 2006. Ecological succession on a natural area in Northeastern Kansas from 1948-2006. Herpetological Conservation and Biology 1:1-5.

FitzSimmons, N.N., C. Moritz, and S.S. Moore. 1995. Conservation and dynamics of microsatellite loci over 300 million years of marine turtle evolution. Molecular Biology and Evolution. 12:432-440.

Flaherty, N. and J.R. Bider. 1984. Physical structures and the social factor as determinants of habitat use by *Graptemys geographica* in southwestern Quebec. American Midland Naturalist 111: 259-266.

Flather, C.H. and Cordell, H.K. 1995. Outdoor recreation: Historical and anticipated trends. *In*: Knight, R.L. and Gutzwiller, K.J. (Eds.). Wildlife and Recreationists: Coexistence through Management and Research. Washington, D.C.: Island Press, pp. 3–16.

Floyd, P. 1973. Singing River sawbacks. International Turtle & Tortoise Society Journal. 7(2): 8-10.

Folkerts, G.W. 1997. State and fate of the world's aquatic fauna. *In* Aquatic fauna in peril: The southeastern perspective. G. W. Benz and D. E. Collins (Eds.). Special Publication 1, Southeast Aquatic Research Institute, Lenz Design and Communication, Decatur, GA. pp. 1-16.

Garber, S.D. and J. Burger. 1995. A 20-year study documenting the relationship between turtle decline and human recreation. Ecological Applications 5(4):1151-1162.

Garza, C. and E.G. Williamson. 2001. Detection of reduction in population size using data from microsatellite loci. Molecular Ecology 10: 305-318.

Gibbons, J.W., D.E. Scott, T.J. Ryan, K.A. Buhlmann, T.D. Tuberville, B.S. Metts, J.L. Greene, T. Mills, Y. Leiden, S. Poppy, and C.T. Winne. 2000. The global decline of reptiles, deja vu amphibians. Bioscience 50(8): 653-666.

Gibbs, J.P. and G.D. Amato. 2000. Genetics and demography in turtle conservation. *In* Turtle Conservation. M.W. Klemens (Ed.). Smithsonian University Press, Washington and London. pp. 207-217.

Goudet, J. 1995. FSTAT version 1.2: A computer program to calculate F-statistics. Journal of Heredity 86:485–486.

Gray, E.M. 1995. DNA fingerprinting reveals a lack of genetic variation in northern populations of the western pond turtle (*Clemmys marmorata*). Conservation Biology 9: 1244-1254.

Greenburg, N., and J. Wingfield. 1987. Stress and reproduction: Reciprocal relationships. *In*: D.O. Norris and R. E. Jones (Eds.). Hormones and Reproduction in Fishes, Amphibians, and Reptiles. Plenum Press, New York. pp. 461-503.

Hammond, K.A., J.R. Spotila, and E.A. Standora. 1988. Basking behavior of the turtle *Pseudemys scripta*: Effects of digestive state, acclimation temperature, sex, and season. Physiological Zoology 61: 69-77.

Hauswaldt, J.S. and T.C. Glenn. 2003. Microsatellite DNA loci from the diamondback terrapin (*Malaclemys terrapin*). Molecular Ecology Notes 3: 174-176.

Holland, D.C. 1992. Level and pattern in morphological variation: A phylogeographic study of the western pond turtle (*Clemmy marmorata*). Ph.D. Dissertation. University of Southwestern Louisiana, Lafayette, Louisiana, USA.

Holman, J., L.A. Vøllestad, K.S. Jakobsen, and C.R. Primmer. 2005. Cross-species amplification of zebrafish and central stoneroller microsatellite loci in six other cyprinids. Journal of Fish Biology. 66:851-859.

Horne, B. 1999. Female reproductive ecology and experimental manipulation of nest site vegetation: implications for the conservation of the yellow-blotched map turtle (Graptemys flavimaculata). Thesis, Southeastern Louisiana University. Hammond, Louisiana, USA.

Horne, B., R. Brauman, M. Moore, and R. Seigel. 2003. Reproductive and nesting ecology of the yellow-blotched map turtle, *Graptemys flavimaculata*: Implications for conservation and management. Copeia 2003 (4):729-738.

Howell, F. 2006. Short term impacts of Hurricane Katrina on the dissolved oxygen content of the lower Leaf River water in south Mississippi. Proceedings of the Mississippi Water Resources Board.

IUCN 2008 Redlist. 2008. Summary Statistics for Globally Threatened Species by Taxonomic Group. http://www.iucnredlist.org/static/stats, Table 4a (18 November 2008).

Iverson, J.B. 1991. Patterns of survivorship in turtles (Order Testudines). Canadian Journal of Zoology 69: 385-391.

Jawor, J.M., J.W. McGlothlin, J.M. Casto, T.J. Greives, E.A. Snajdr, G.E. Bentley, and E.D. Ketterson. 2007. Testosterone response to GnRH in a female songbird varies with stage of reproduction: implications for adult behavior and maternal effects. Functional Ecology 21:767-775.

Jensen, J.L., A.J. Bohonak, and S.T. Kelley. 2005. Isolation by distance, web service. BMC Genetics (6): 13.

Jones, M.E., D. Paetkau, E. Geffen, and C. Moritz. 2004. Genetic diversity and population structure of Tasmanian devils, the largest marsupial carnivore. Molecular Ecology 13: 2197-2209.

Jones, R.L. 1994. Density, population structure, and movements of the yellow-blotched map turtle (*Graptemys flavimaculata*). Mississippi Museum of Natural Science Technical Report No. 30. 65 pp.

Jones, R.L. 1996**.** Home range and seasonal movements of the turtle *Graptemys flavimaculata*. Journal of Herpetology 30: 376-385.

Jones, R.L. and W. Selman. 2009. *Graptemys oculifera* Baur 1890- Ringed Map Turtle, Ringed Sawback. *In*: Rhodin, A.G.J., Pritchard, P.C.H., van Dijk, P.P., Saumere, R.A., Buhlmann, K.A., and Iverson, J.B. (Eds.). Conservation Biology of Freshwater Turtles and Tortoises: A Compilation Project of the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group. Chelonian Research Monographs No. 5, pp. 029.1-029.8, doi:10.3854/crm.5.033.oculifera.v1.2009, http://www.iucn-tftsg.org/cbftt/.

Jones, R.L., W.T. Slack, and P.D. Hartfield. 2005. The freshwater mussels (Mollusca: Bivalvia: Unionidae) of Mississippi. Southeastern Naturalist 4(1):77-92.

Ketterson, E.D., V. Nolan Jr., L. Wolf, C. Ziegenfus, A.M. Dufty, G.F. Ball, and T.S. Johnsen. 1991. Testosterone and avian life histories: The effect of experimentally elevated testosterone on corticosterone and body mass. Hormones and Behavior 25:489-503.

King, T.L. and S.E. Julian. 2004. Conservation of microsatellite DNA flanking sequence across 13 Emydid genera assayed with novel bog turtle (*Glyptemys muhlenbergii*) loci. Conservation Genetics 5: 719-725.

Klemens, M.W (Ed.). 2000. Turtle Conservation. Washington and London: Smithsonian Institute Press.

Krawchuk, M.A. and R.J. Brooks. 1998. Basking behavior as a measure of reproductive cost and energy allocation in the painted turtle, *Chrysemys picta*. Herpetologica 54 (1): 112-121.

Krzysik, A. J. 1997. Desert tortoise populations in the Mojave Desert and a half-century of military training activities. *In* Proceedings: Conservation, Restoration, and Management of Tortoises and Turtles: An International Conference. J. Van Abbema (Ed.). New York Turtle and Tortoise Society, New York. pp. 61-73.

Lamb, T., C. Lydeard, R.B. Walker, and J.W. Gibbons. 1994. Molecular systematics of map turtles (*Graptemys*): A comparison of mitochondrial restriction site versus sequence data. Systematic Biology 43: 534-559.

Licht, P. 1984. Reptiles. *In* Marshall's Physiology of Reproduction. G.E. Lamming (Ed.). Churchill, Edinburgh, London. pp. 206-282.

Licht, P., G.L. Breitenbach, and J.D. Congdon. 1985. Seasonal cycles in testicular activity, gonadotropin, and thyroxine in the painted turtle, *Chrysemys picta*, under natural conditions. General Comparative Endocrinology. 59:130-139.

Lindeman, P.V. 1993. Aerial basking by hatchling freshwater turtles. Herpetological Review 24: 84-87.

Lindeman, P.V. 1998. Of deadwood and map turtles (*Graptemys*): An analysis of species status for five species in three river drainages using replicated spotting-scope counts of basking turtles. Linnaeus fund research report. Chelonian Conservation and Biology 3(1):137-141.

Lindeman, P.V. 1999. Surveys of basking map turtles *Graptemys* spp. in three river drainages and the importance of deadwood abundance. Biological Conservation 88: 33-42.

Lindeman, P.V. and M.J. Sharkey. 2001. Comparative analyses of functional relationships in the evolution of trophic morphology in the map turtles (Emydidae: *Graptemys*). Herpetologica 57:313-318.

Lovich, J.E., Selman, W., and McCoy, C.J. 2009. *Graptemys gibbonsi* Lovich and McCoy 1992 – Pascagoula map turtle, Pearl River map turtle, Gibbons' map turtle. *In*: Rhodin, A.G.J., Pritchard, P.C.H., van Dijk, P.P., Saumure, R.A., Buhlmann, K.A., Iverson, J.B., and Mittermeier, R.A. (Eds.). Conservation Biology of Freshwater Turtles and Tortoises: A Compilation Project of the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group. Chelonian Research Monographs No. 5, pp. 029.1-029.8, doi:10.3854/crm.5.029.gibbonsi.v1.2009, http://www.iucn-tftsg.org/cbftt/.

MacCulloch, R.D. and D.M. Secoy. 1983. Movement in a river population of *Chrysemys picta bellii* in southern Saskatchewan. Journal of Herpetology 17: 283-285.

Mahmoud, I.Y., L.J. Guillette., Jr., M.E. McAsey, and C. Cady. 1989. Stress-induced changes in serum testosterone, estradiol-17β, and progesterone in the turtle, *Chelydra serpentina*. Comparative Biochemical Physiology (A) 93:433-427

Mallin, M.A., M.H. Posey, G.C. Shank, M.R. McIver, S.H. Ensign, and T.D. Alphin. 1999. Hurricane effects on water quality and benthos in the Cape Fear watershed: Natural and anthropogenic impacts. Ecological Applications 9(1): 350-362.

Manning, B. and G.C. Grigg. 1997. Basking is not of thermoregulatory significance in the "basking" freshwater turtle *Emydura signata*. Copeia 1997: 579-584.

McCoy, C.J., and R.C. Vogt. 1980. Distribution and population status of the ringed sawback turtle (*Graptemys oculifera*), blotched sawback (*Graptemys flavimaculata*), and black-knobbed sawback (*Graptemys nigrinoda*) in Alabama and Mississippi. 30 pp. USFWS Contract No. 14-16-0004-79-038.

Mickle, P.F., J.F. Schaefer, S.B. Adams, and B.R. Kreiser. 2009. Habitat use of age 0 Alabama Shad in the Pascagoula River drainage, USA. Ecology of Freshwater Fish 19(1): 107-115.

Mississippi Department of Wildlife, Fisheries, and Parks. 2005. Mississippi's comprehensive wildlife conservation strategy. Version 1.1. 11 p.

Mississippi Natural Heritage Program. 2003. Endangered Species of Mississippi. Museum of Natural Science, Mississippi Department of Wildlife, Fisheries, and Parks, Jackson, MS. 2pp.

Mitchell, J.C. and M.W. Klemens. 2000. Primary and secondary effects of habitat alteration. *In* Turtle Conservation. M.W. Klemens (Ed.). Smithsonian Institute Press, Washington and London. pp. 5-32.

Moberg, G.P. 1985. Influence of stress on reproduction, measure of well-being. *In* Animal Stress. G.P. Moberg (Ed.). American Physiological Society, Bethesda, MD. pp. 246-267

Moll, D. and E.O. Moll. 2004. The ecology, exploitation, and conservation of river turtles. Oxford University Press, New York, New York.

Moll, E.O. 1982. Freshwater turtle trade: Drugs. IUCN/SSC Freshwater Chelonian Specialist Group Newsletter (3):1-2.

Moll, E.O. and J.M. Legler 1971. The life history of the neotropical slider, *Pseudemys scripta* (Schoepff), in Panama. Bulletin of the Los Angeles County Museum Natural History 11: 1-102.

Monagas, W.R. and R.E. Gatten. 1983. Behavioral fever in turtles *Terrapene carolina* and *Chrysemys picta*. Journal of Thermal Biology 8:285-288.

Moore, M.J.C. 2003. Behavioral ecology of the threatened yellow-blotched map turtle (*Graptemys flavimaculata*): Implication for conservation and management. Thesis, Southeastern Louisiana University, Hammond, Lousiana, USA.

Moore, M.J.C. and R.A. Seigel. 2006. No place to nest or bask: Effects of human disturbance on the nesting and basking habits of yellow-blotched map turtles (*Graptemys flavimaculata*). Biological Conservation 130: 386-393.

Mount, R.H. 1975. The reptiles and amphibians of Alabama. Auburn University Agricultural Experiment Station, Auburn, Alabama, USA.

Neill, W.T. and E.R. Allen. 1954. Algae on Turtles: Some Additional Considerations. Ecology 35: 581-584.

Otvos, E.G. In Press. Geological Framework and Evolution History. *In* Grand Bay National Estuarine Research Reserve: An Ecological Characterization M.S. Peterson, G.L. Waggy and M.S. Woodrey (Eds.). Grand Bay National Estuarine Research Reserve, Moss Point, Mississippi. pp. 22 – 47.

Peakall, R., Smouse, P.E., 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6: 288-295.

Pearse, D.E., A.D. Arndt, N. Valenzuela, B.A. Miller, V. Cantarelli, and J.W. Sites Jr. 2006. Estimating population structure under nonequilibrium conditions in a conservation context: continent-wide population genetics of the giant Amazon river turtle, *Podocnemis expansa* (Chelonia, Podocnemididae). Molecular Ecology 15: 985-1006.

Piry, S., G. Luikart, and J.M. Cornuet. 1999. BOTTLENECK: A computer program for detecting recent reductions in the effective population size using allele frequency data. Journal of Heredity 90: 502-503.

Primmer, C.R., A.P. Møller, and H. Ellegren. 1996. A wide-range survey of cross-species microsatellite amplification in birds. Molecular Ecology 5:365-378.

Pritchard, J.K., M. Stephens, and P. Donnelly. 2000. Interence of population structure using multilocus genotype data. Genetics 155: 945-959.

Pritchard, P.C.H. and W. Greenhood 1968. The sun and the turtle. International Turtle and Tortoise Society Journal 2: 20-25, 34.

Raymond, M. and F. Rousett. 1995. GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248-249.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution 263:223-225.

Rico, C., I. Rico, and G. Hewitt. 1996. 470 million years of conservation of microsatellite loci among fish species. Proceedings of the Royal Society of London, B 263:549-557.

Ross, S.T. 2001. The inland fishes of Mississippi. University Press of Mississippi, Jackson, Mississippi, USA. 624 pp.

Sapolsky, R.M., L.M. Romero, and A.U. Munck. 2000. How do glucocorticoids influence stress response? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocrine Reviews 21(1):55-89.

Sanderson, R.A. 1974. Sexual dimorphism in the Barbour's map turtle, *Malaclemys barbouri* (Carr and Marchand). Thesis, University of South Florida, Tampa, Florida, USA.

Schaefer, J., P. Mickle, J. Spaeth, B. Zuber, W. Matamoros, S. Adams, B. Kreiser, and P. Vigueira. 2006. Effects of Hurricane Katrina on the fish fauna of the Pascagoula drainage. Proceedings of the Mississippi Water Resources Board 36:62-68.

Seber, G.A.F. 1982. The estimation of animal abundance and related parameters. 2nd ed. Charles Griffin & Co. Ltd., London, U.K.

Seigel, R. and R. Brauman. 1994. Food habits of the yellow-blotched map turtle (*Graptemys flavimaculata*). Report to the Mississippi Department of Wildlife, Fisheries, and Parks. Jackson, MS. 18 pp.

Selman, W. and R.L. Jones. 2010. *Graptemys flavimaculata* Cagle 1954- Yellow-blotched Sawback, Yellow-blotched Map Turtle. *In*: Rhodin, A.G.J., Pritchard, P.C.H., van Dijk, P.P., Saumere, R.A., Buhlmann, K.A., and Iverson, J.B. (Eds.). Conservation Biology of Freshwater Turtles and Tortoises: A Compilation Project of the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group.

Selman, W. and C. Qualls. 2005. Steroid hormone levels and reproduction in the yellow-blotched sawback turtle (*Graptemys flavimaculata*) and the congeneric ringed sawback turtle (*Graptemys oculifera*). Report to the Mississippi Department of Wildlife, Fisheries, and Parks. Jackson, MS. 33 pp.

Selman, W. and C. Qualls. 2006. Steroid hormone levels and current population status of the yellow-blotched sawback turtle (*Graptemys flavimaculata*). Technical report to the Mississippi Department of Wildlife, Fisheries, and Parks. Jackson, MS. 58 pp.

Selman, W. and C. Qualls. 2008. The impacts of Hurricane Katrina on a population of yellow-blotched sawbacks (*Graptemys flavimaculata*) in the Lower Pascagoula River. Herpetological Conservation and Biology 3(2):224-230.

Selman, W. and C. Qualls. 2009a. *Graptemys flavimaculata* (Yellow-blotched Map Turtle). Basking and parasite removal. Herpetological Review 40(1): 78-79.

Selman, W. and C. Qualls. 2009b. Distribution and Abundance of Two Imperiled *Graptemys* Species of the Pascagoula River System. Herpetological Conservation and Biology. Herpetological Conservation and Biology 4(2): 171-184.

Selman, W. and C. Qualls. 2009c. *Pseudemys concinna* (River Cooter). Maximum basking time. Herpetological Review 40(1): 79-80.

Selman, W. and C. Qualls. 2009d. *Trachemys scripta elegans* (Red-Eared Slider). Maximum basking time. Herpetological Review 40(1): 80.

Selman, W., D. Strong, and C. Qualls. 2008. *Graptemys gibbonsi* (Pascagoula Map Turtle). Basking and parasite removal. Herpetological Review 39(2): 216.

Selman, W., D. Strong, and C. Qualls. 2009. *Graptemys flavimaculata* (Yellow-blotched Map Turtle). Unusual basking disturbance. Herpetological Review 40(1): 78.

Selman, W., J. Ennen, B. Kreiser, and C. Qualls. 2009b. Cross–species amplification of Emydid microsatellite loci in three *Graptemys* species and their utility as a conservation tool. Herpetological Review 40(1): 32-37.

Shealy, R.M. 1976. The natural history of the Alabama map turtle, *Graptemys pulchra* Baur, in Alabama. Bulletin of the Florida State Museum, Biological Science 21: 47-111.

Shelby, J.A., M.T. Mendonça, B.H. Horne, and R.A. Seigel. 2000. Seasonal variation in reproductive steroids of male and female yellow-blotch map turtles, *Graptemys flavimaculata*. General and Comparative Endocrinology 119: 43-51.

Shelby, J.A., and M.T. Mendonça. 2001. Comparison of reproductive parameters in male yellow-blotched map turtles (*Graptemys flavimaculata*) from a historically contaminated site and a reference site. Comparative Biochemistry and Physiology Part C 129: 233-242.

Shively, S.H. 1999. Survey for the ringed map turtle (*Graptemys oculifera*) in the Bogue Chitto River, Louisiana. Unpublished report to the Louisiana Natural Heritage Program and the Louisiana Department of Wildlife and Fisheries. Baton Rouge, LA. 50 pp.

Shively, S.H. and J.F. Jackson. 1985. Factors limiting the upstream distribution of the Sabine map turtle. American Midland Naturalist 114: 292-303.

Spinks, P.Q. and H.B. Shaffer. 2005. Range-wide molecular analysis of the western pond turtle (*Emys marmorata*): Cryptic variation, isolation by distance, and their conservation implications. Molecular Ecology 14: 2047-2064.

Starkey, D.E., H.B. Shaffer, R.R. Burke, M.R.J. Forstner, J.B. Iverson, F.J. Janzen, A.G.J. Rhodin, and G.R. Ultsch. 2003. Molecular systematics, phylogeography, and effects of Pleistocene glaciation in the painted turtle (*Chrysemys picta*) complex. Evolution. 57: 119-128.

Stewart, J.H. 1989. Status review of yellow-blotched map turtle, *Graptemys flavimaculata*. Unpublished report to the U.S. Fish and Wildlife Service. Jackson, MS.

Thiel, D, S. Jenni-Eiermann, V. Braunisch, R. Palme, and L. Jenni. 2008. Ski tourism affects habitat use and evokes a physiological stress response in capercaillie *Tetrao urogallus*: A new methodological approach. Journal of Applied Ecology 45: 845-953.

Thorbjarnarson, J., C.J. Lagueux, D. Bolze, M.W. Klemens, and A.B. Meylan. 2000. Human use of turtles: A worldwide perspective. *In* Turtle Conservation. M.W. Klemens (Ed.). Smithsonian Institute Press, Washington and London. pp. 33-84.

Tinkle, D.W. 1958. The systematics of the Sternothaerus carinatus complex (Testudinata, Chelydridae). Tulane Stud. Zoology 6:3-56.

U.S. Fish and Wildlife Service. 1991. Endangered and threatened wildlife and plants: threatened status for the yellow-blotched map turtle, *Graptemys flavimaculata*. Federal Register 56: 1459-1463.

U.S. Fish and Wildlife Service. 1993. Yellow-blotched map turtle (*Graptemys flavimaculata*) recovery plan. U.S. Fish and Wildlife Service. Jackson, Mississippi, USA. 18 p.

Van Dijk, P.P. 2000. The status of turtles in Asia. *In* Asian Turtle Trade: Proceedings of a workshop on conservation and trade of freshwater turtles and tortoises in Asia. Vol. 2. P.P. van Dijk, B.L. Stuart, and A.G.J. Rhodin (Eds.). Chelonian Research Foundation, Phnom Penh, Cambodia. p.15-23.

Van Dijk, P.P., B.L. Stuart, and A.G.J. Rhodin. 2000. Asian turtle trade: Proceedings of a workshop on Conservation and trade of freshwater turtles and tortoises in Asia. *In* Chelonian Research Monographs. Vol. 2. P.P. van Dijk, B.L. Stuart, and A.G.J. Rhodin (Ed.). Chelonian Research Foundation, Phnom Penh, Cambodia. 164 p.

Viveiros, M.M. and R. M. Liptrap. 1995. Altered ovarian follicle function in ACTH-treated gilts. Animal Reproduction Science 40: 107-119.

Vogt, R.C. 1979. Cleaning/feeding symbiosis between grackles (*Quiscalus*: Icteridae) and map turtles (*Graptemys*: Emydidae). Auk 96: 609.

Vogt, R.C. 1980. Natural history of the map turtles *Graptemys pseudogeographica* and *G. ouachitensis* in Wisconsin. Tulane Studies in Zoology and Botany 22: 17-48.

von Hildebrand, P., N. Bermudez, M.M.C. Peñuela. 1997. La Tortuga Charapa (*Podocnemis expansa*) en el Río Caquetá, Amazonas, Columbia. Aspectos de la Biología Reproductiva y Técnicas para su Manejo. Bogatá, Colombia, Disloque.

Walker, B.G., P. D. Boersma, and J.C. Wingfield. 2005. Physiological and behavioral differences in Magellanic Penguin chicks in undisturbed and tourist-visited locations of a colony. Conservation Biology 19(5): 1571-1577.

Walker, B.G., P. D. Boersma, and J.C. Wingfield. 2006. Habituation of adult Magellanic Penguins to human visitation as expressed through behavior and corticosterone secretion. Conservation Biology 20(1): 146-154.

Waters, J.C. 1974. The biological significance of the basking habit in the black-knobbed sawback, *Graptemys nigrinoda* Cagle. Thesis, Auburn University, Auburn, Alabama, USA.

Weathers, W.W. and F.N. White. 1971. Physiological thermoregulation in turtles. American Journal of Physiology 221: 704-710.

White, G.C. 1996. NOREMARK: Population estimation from mark-resighting surveys. Wildlife Society Bulletin 24:50–52.

Williams, J.D., A.E. Bogan, and J.T. Garner. 2008. Freshwater Mussels of Alabama and the Mobile Basin in Georgia, Mississippi and Tennessee. University of Alabama Press, Tuscaloosa, Alabama, USA.

Wingfield, J. C., and D.S. Farner. 1975. The determination of five steroids in avian plasma by radioimmunoassay and competitive protein binding. Steroids 26:311-327.